FIRST EUROPEAN MEETING ON GLIAL CELL FUNCTION IN HEALTH AND DISEASE

HEIDELBERG
MARCH 24 - 27, 1994

ACKNOWLEDGEMENT

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NOTES

Meeting site: Auditorium "Physik", Im Neuenheimer Feld 308, D-69120 Heidelberg (see map on back cover)

Office Hours:	Thursday, March 24	15.00 - 20.00
	Friday, March 25	8.00 - 20.00
	Saturday, March 26	8.00 - 20.00
	Sunday, March 27	8.30 - 13.00
Office Phone:	(49) 6221 56 4900	
Fax:	(49) 6221 56 3801	

Registration: Please register after your arrival at the registration desk.

Posters: The size of the poster boards is 90 cm (width) x 110 cm (height). Do not forget to bring pins to fix the poster.
The poster boards are numbered.
The poster sessions on Friday are reserved for the posters No. 1 - 160, and the poster sessions on Saturday are reserved for the posters No. 161 - 320. In the morning sessions posters with odd numbers will be presented, in the afternoon, posters with even numbers will be presented (see "Poster Presentations", pp. 49 - 195).

Poster Session I: Posters No. 1 - 159, odd numbers
Poster Session II: Posters No. 2 - 160, even numbers
Poster Session III: Posters No. 161 - 319, odd numbers
Poster Session IV: Posters No. 162 - 320, even numbers

Meals: On Friday, March 25, the University Cafeteria serves lunch from 11.30 - 14.00. On Saturday, March 26, lunch is available at the meeting site.

ORGANIZATION

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PROGRAMME

Thursday March 24th, 1994

15.00 - 18.00 **Registration**

18.00 - 19.00 **Key Note Feature** - Chairperson: Rhona Mirsky

Nicole M. Le Douarin, Insitut d'Embryologie cellulaire et moléculaire du CNRS et du Collège de France, Nogent-sur- Marne, France
Differentiation of glial cells in the peripheral nervous system studied in the avian embryo

19.00 **Get-together at the Cold Buffet**

Friday March 25th, 1994

9.00 - 10.00 **Key Note Lecture -** Chairperson: Andreas Faissner

Eric M. Shooter, Department of Neurobiology, Stanford University, Stanford, USA
The molecular basis of peripheral neuropathies in mouse and human

10.00 - 11.00 **1. Session: Development of Glial Cells -** Chairpersons: Bernard Zalc and Eva Sykova

Kristjan Jessen, Department of Anatomy, University College London, UK
Schwann Cell precursors in the rat: survival and development

Jack Price, Department of Molecular Neuropathology, Smith Kline Beecham Pharmaceuticals, Essex, UK
The generation of cellular diversity in the rat cerebral cortex

11.00 - 13.00 **Poster Session I**

13.00 - 14.00 **Lunch**

14.00 - 15.30 **Chairpersons: Giulio Levi and Jacqueline Trotter**

Martin Raff, Department of Neurobiology Programme, MRC, University College London, UK
Controlling oligodendrocyte numbers

Christo Goridis, Laboratoire de Génétique et Physiologie du Développement CNRS, Université Aix-Marseille II, France
A possible link between cell adhesion receptors, homeodomain transcription factors and neuronal identity

Marc Noble, Ludwig Institute for Cancer Research, London, UK
From glial precursors to glial tumors

15.30 - 17.30 **Poster Session II**

17.30 - 20.00 **2. Session: Physiological aspects of neuroglial interactions** - Chairpersons: Jonathan Coles and Jochen Deitmer

Marco Tsacopoulos, Experimental Ophthalmology Laboratory, Medical Research Center, Geneva, Switzerland
Metabolic interactions and signal trafficking between glial cells and neurons

Murdoch Ritchie, Department of Pharmacology, Yale University, New Haven, USA
Voltage-gated K^+ channels in rabbit Schwann cells

Steven Finkbeiner, Department of Neurology, University of California, San Francisco, USA
Temporal and spatial aspects of calcium signalling in cultured rat hippocampal astrocytes

Ken McCarthy, Department of Pharmacology, University of North Carolina, Chapel Hill, USA
Receptor-mediated changes in astroglial calcium levels: integration of signals and "action potential like" calcium responses

Hervé Chneiweiss, INSERM U 114, Paris, France
Multiple phosphorylation cascades in astrocytes triggered by neurotransmitters and growth factors: studies using PEA-15, a novel major astrocytic phosphoprotein, as a probe

Saturday March 26th, 1994

9.00 - 10.30 **3. Session: Myelin Forming Cells** - Chairpersons: Elisabeth Hansson and Dionysia Theodosis

Martin E. Schwab, Brain Research Institute, University of Zürich, Switzerland
The role of oligodendrocytes for the maturation, stabilization and regeneration of the central nervous system

A. Baron-Van Evercooren, INSERM U134, Hôpital de la Salpêtrière, Paris, France
Migration of myelin forming cells in the adult demyelinated spinal cord

Peter J. Brophy, Department of Biological and Molecular Sciences, University of Stirling, Scotland
Periaxin, a novel cytoskeletal protein of myelinating Schwann cells

10.30 - 12.30 **Poster Session III**

12.30 - 14.00 **Lunch**

14.00 - 16.00

4. Session: Growth factors, cell adhesion molecules and extracellular matrix proteins - Chairpersons: Gustave Moonen and Monique Sensenbrenner

Denis Monard, Friedrich Miescher Institute, Basel, Switzerland
Neuropeptides as regulators of protease nexin-1 expression in cultured Schwann cells

Yves-A. Barde, Abteilung Neurochemie, Max-Planck-Institut für Psychiatrie, Martinsried, Germany
Control of cell numbers by neurotrophins

Klaus Unsicker, Department of Anatomy, University of Heidelberg, Germany
Growth factors and astroglial cells: focus on FGFs, TGFßs and CNTF

Melitta Schachner, Neurobiology, ETH-Hönggerberg, Zürich, Switzerland

16.00 - 17.30

Poster Session IV

17.30 - 19.30

5. Session: Interaction of glial cells with their environment - Chairpersons: Paul Honegger and Marcienne Tardy

Werner Risau, Max-Planck-Institut für physiologische und klinische Forschung, Bad Nauheim, Germany
Glia and the blood-brain barrier

Georges E. Grau, Department of Pathology, CMU, University of Geneva, Switzerland
Platelet fusion of endothelial cells: a critical mechanism of blood-brain barrier changes induced by TNF-α

Bernd Hamprecht, Physiologisch-Chemisches Institut, Universität Tübingen, Germany
The function of astrocyte glycogen

Jean de Vente, Department of Psychiatry, Limburg University, The Netherlands
The nitric oxide synthase - cGMP connection in the rat brain and the involvement of glial cells

19.30

Round Table Discussion on a Future European Network with the Coordinators from the European Countries
Chairpersons: Nicole Baumann and Helmut Kettenmann

Sunday March 27th, 1994

9.00 - 10.00 **6. Session: Glial Involvement in Brain Pathology** - Chairpersons: Christine Dijkstra and Graham Wilkin

Georg W. Kreutzberg, Max-Planck-Institute for Psychiatry, Martinsried, Germany
Microglia, the first line of defense in the CNS

Hans Lassmann, Research Unit of Experimental Neuropathology, Austrian Academy of Sciences, Vienna, Austria
Oligodendrocytes in multiple sclerosis

10.00 - 10.30 **Coffee Break**

10.30 - 12.00 Chairpersons: Rolf Heumann and Joachim Wolff

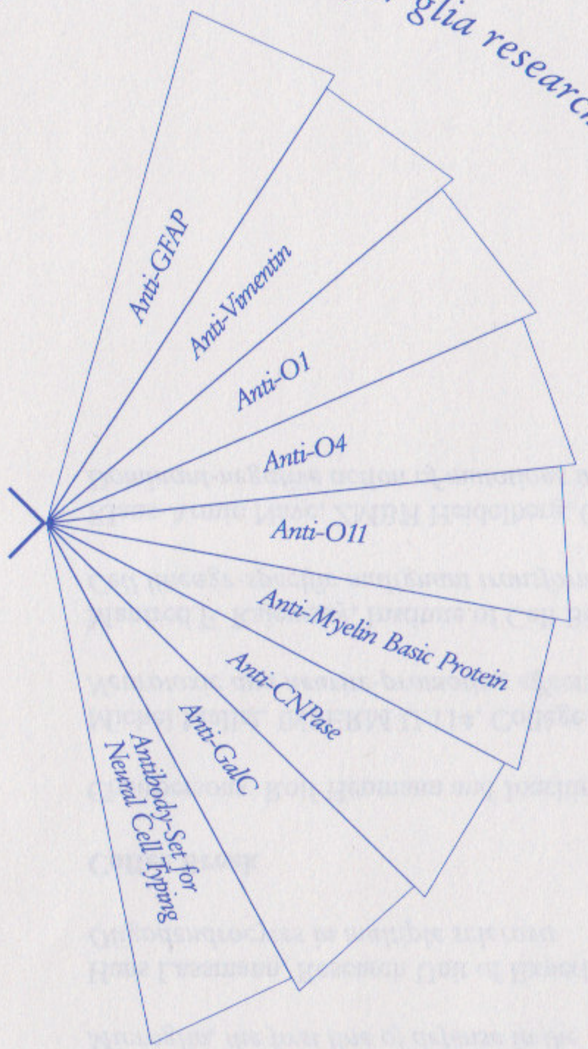
Michel Mallat, INSERM U.114, Collège de France, Paris, France
Neurotoxic and neurite-promoting effects of brain macrophages

Manfred F. Rajewsky, Institute of Cell Biology, University of Essen, Germany
Cell lineage-specific malignant transformation in the developing nervous system of the rat

Klaus-Armin Nave, ZMBH Heidelberg, Germany
Dominant-negative action of mutations in the myelin proteolipid protein (PLP/DM-20) gene

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POSTER PRESENTATIONS

1. Aksentsev, Sergej L., Mongin, A. A., Orlov, S. N., Slepko, N. G., and Konev, S. V., Institute of Photobiology, Belarus Academy of Sciences, Minsk, Belarus
The effect of calmodulin antagonist R24571 on swelling-induced K^+ efflux and influx in C6 glioma cells

2. Aloisi, F., De Simone, R., Ceccerini, F., Rosa, S., Testa, U., Peschle, C., and Levi G., Istituto Superiore di Sanita, Rome, Italy
Regulation of leukemia inhibitory factor, platelet-derived growth factor and fibroblast growth factor synthesis in cultured human astrocytes

3. Althaus, H. H., Kruska, L., Klöppner, S., and Heumann, R., Max-Planck-Institute for Experimental Medicine, Göttingen, Germany
Oligodendrocytes isolated from pig or rat, are both target cells for growth factors and cytokines, but respond to a different spectrum

4. Alvarez, Jaime, Moreno, R. C., and Inestrosa, N. C., Departamento de Biologia Celular y Molecular, Universidad Catolica, Santigo, Chile
Mitosis of Schwann cells induced in vivo by APP and other protease inhibitors

5. Amédée, T. and Despeyroux, S., INSERM U394, Neurobiologie Intégrative, Bordeaux Cedex, France
Membrane responses to externally applied ATP in mouse Schwann cells from organotypic culture of dorsal root ganglia

6. Arcuri, C., Bocchini, V., and Tardy, M., Cellular Biology Section, University of Perugia, Italy
Effects of PKC and PKA stimulation on cell morphology, GFAP and GS expression in a glioblastoma multiform cell line of clonal origin

7. Asmal, E., and Omlin, F. X., Department of Anatomy and Cell Biology, Medical School, University of Cape Town, South Africa
Cytosine arabinoside induces structural changes in developing postnatal rat optiv nerves

8. Avola, R., Spina Purrello, V., Reale, S., Costa, A., Lalicata, C., Ragusa, N., and Giuffrida Stella, A. M., Institute of Biochemistry, University of Catania, Italy
ADP-ribosylation process of nuclear proteins in glial cell cultures and fetal rat brain tissue slices treated with growth factors

9. Baas, D., Louis, J. C., Puymirat, J., and Sarlième, L. L., Centre de Neurochimie du CNRS, Strasbourg, France
Temporal expression of ß1 and α1 thyroid receptors in the bipotential cell line, central glia (CG-4)

10. Bal, Anna, Bachelot, T., Savasta, M., Manier, M., Verme, J. M., Benabid, A. L., and Feuerstein, C., Dept. of Neurosciences, INSERM U 318, CHU de Grenoble, France
Evidence for dopamine D2 receptor mRNA expression by striatal astrocytes in culture; in situ hybridization and polymerase chain reaction studies

11. Ballerini, P., Ciccarelli, P., Di Iorio, P., Ambrosini, G., Giuliani, P., Tiboni, G., and Caciagli, F., Inst. of Pharmacology and Bio-Medical Tech., University of Chieti, Italy
Purine release from rat glial cultures is modulated by agents affecting cell Ca^{2+} availability

12. Baltrons, M. A., Agullo, L., and Garcia, A., Instituto Biologia Fundamental, Universidad Autônoma de Barcelona, Spain
Regulation of nitric oxide-dependent cGMP formation in cerebellar glial cells

13. Barac-Latas, V., Wege, H., and Lassmann, H., Neurologisches Institut, Universität Wien, Austria
The fate of oligodendroglia in coronavirus induced demyelinating encephalomyelitis

14. Barnett, Susan C., Department of Neurology/Medical Oncology, University of Glasgow, UK
Analysis of FOS and JUN family members in the growth and differentiation of the oligodendrocyte-type-2 astrocyte (0-2A) cell lineage

15. Bauer, J., Berkenbosch, F., Van Dam, A.- M., and Dijkstra, C., Department of Cell Biology, Vrije Universiteit, Amsterdam, The Netherlands
Interleukin-1ß in Lewis rat brain during experimental autoimmune encephalomyelitis is present in microglial cells but not in astrocytes

16. Berger, Thomas and Backus, K. H., Institute of Anatomy, University of Freiburg, Germany
Voltage-gated currents of glial cells in the juvenile rat hilus

17. Bernstein, M., and Kettenmann, H., Max-Delbrück-Center for Molecular Medicine, Berlin-Buch, Germany
Single channel properties in glial precursor cells of the mouse corpus callosum slice

18. Beslin, A., Blondeau, J. P., Chantoux, F., and Francon, J., INSERM U96, Le Kremlin-Bicêtre Paris, France
Transmembrane and intracellular transport of thyroid hormones in cultured astrocytes

19. Biedermann, B., and Reichelt, W., Carl-Ludwig-Institute of Physiology, Unversity of Leipzig, Germany
GABA uptake into isolated Müller glial cells of the guinea pig retina

20. Bielinsky, A. K., Nietzgen, B., Hadding, U., and Fischer H. G., Institute for Medical Microbiology and Virology, University of Düsseldorf, Germany
Astrocytic M-CSF as cofactor required by microglia for IFNγ-independent antigen presentation

21. Biffo, S., Dechant, G., and Barde, Y. - A., Department of Neurochemistry, Max-Planck-Institute for Psychiatry, Martinsried, Germany
Non-neuronal expression of the BDNF receptor truncated *trk*B

22. Birling, M.- C., Miéhé, M., Nussbaum, F., and Nussbaum, J.- L., Laboratoire de Neurobiologie Ontogénique, CNRS, Strasbourg, France
A new oligodendrocyte specific plasma membrane surface protein identified by a monoclonal antibody produced *in vitro*

23. Bixel, M. G., Dringen, R., Wiesinger, H., Stock, W., and Hamprecht, B.. Physiological-Chemical Institute, University of Tübingen, Germany
Consumption of branched-chain amino acids and glycine by astroglia-rich rat brain cell cultures

24. Blakemore, W. F., Crang, A. J., and Ryder, S. R., Department of Clinical Veterinary Medicine, University of Cambridge, UK
Areas of demyelination repaired by mouse oligodendrocytes and rat type-1 astrocytes create a new model of immune-mediated demyelination

25. Boersma, C. J. C., van Leeuwen, F. W., Mason, W. T., and Bicknell, R. J., Netherlands Institute for Brain Research, Amsterdam, The Netherlands
Dynorphin 1-17 delays the vasopressin induced mobilization of intracellular calcium in cultured astrocytes from the rat neural lobe

26. Borges, K., Ohlemeyer, C., Trotter, J., and Kettenmann, H., Department of Neurobiology, University of Heidelberg, Germany
AMPA/kainate receptor activation in murine oligodendrocyte precursor cells leads to activation of a cation conductance, CA^{2+} influx and K^{+} channel blockade

27. Borges, K., Wolswijk, G., Ohlemeyer, and Kettenmann, H., Department of Neurobiology, University of Heidelberg, Germany
Adult rat oligodendrocyte progenitor cells express a repertoire of voltage and ligand gated ion channels distinct from their perinatal counterparts

28. Boulland, M. L., Fages, C., Rolland, B., and Tardy, M., INSERM U282, Hôpital Henri Mondor, Creteil, France
Lipopolysaccharides induce an up-regulation of the IL-1 mRNA and a down-regulation of the GFAP-mRNA in cultured mouse astroglial cells

29. Boussouf, A., and Gaillard, S., Laboratoire de Neurobiologie Cellulaire, CNRS, Strasbourg, France
Regulation of intracellular pH in cultured rat oligodendrocytes: evidence for Na^+/H^+ and HCO_3^- dependent acid extrusion mechanisms

30. Brennan, A., Gavrilovic, J., Mirsky, R., and Jessen, K. R., Department of Anatomy, University College London, UK
Fibroblast growth factors and insulin-like growth factors support Schwann cell precursors *in vitro*

31. Brichová, H., Institute of Histology and Embryology, Charles University, Prague, Czech Republic
Differentiation of rat telencephalon glia cells under hypoxia conditions

32. Bröer, S., Bröer, A., and Hamprecht, B., Physiologisch-Chemisches Institut, Universität Tübingen, Germany
Expression of a sodium independent neutral amino acid transporter from astroglia cells in *Xenopus laevis* oocytes

33. Brückner, G., Welt, K., Brauer, K., Härtig, W., and Bigl, V., Paul Flechsig Institute for Brain Research, University of Leipzig, Germany
Regional peculiarities of glial-matrix-neuron interface in the rat brain

34. Brune, T., Fetzer, S., Backus, K. H., and Deitmer, Joachim W., Abteilung für Allgemeine Zoologie, Universität Kaiserslautern, Germany
Intracellular pH regulation in cultured rat cerebellar astrocytes

35. Brunet, Jean-Francois M., Berger, Francois, Lainé, Michèle, and Benabid, Alim Louis, Laboratoire de Neurobiophysique, INSERM U318, Grenoble, France
Antibody activities in glioma cyst fluids

36. Bureau, J. F., Azoulay, A., Bihl, F., Lefebvre, S., Montagutelli, X., Aguet, M., Guénet, J. L. and Brahic, M., Unité des Virus Lents, Institut Pasteur, Paris, France
Genetic susceptibility of the mouse to the persistent infection by Theiler's virus

37. Butt, Arthur M. and Jennings Jason, Department of Physiology, UMDS, St- Thomas's Hospital, London, UK
Astrocyte response to GABA in the developing rat optic nerve

38. Butt, Arthur M., Duncan, Al, Colquhoun, K., and Berry, M., Departments of Physiology and Anatomy, UMDS, St- Thomas's Hospital, London, UK
Astrocyte association with nodes of Ranvier in the mouse optic nerve

39. Buttini, M., Sauter, A., and Boddeke, H. W. G. M., Preclinical Research, Sandoz Ltd., Basle, Switzerland
Induction of interleukin-1ß mRNA after focal cerebral ischemia in the rat

40. Calka, J., Schmidt, W., and Wolf, G., Institute of Medical Neurobiology, University of Magdeburg, Germany,
Activated microglia express nitric oxide synthase (NOS) activity in quinolinate lesioned rat striatum: an ultrastructural study

41. Cardinaud, B., Coles, J. A., Perrottet, P., Spencer, A., Osborne, M. P., and Tsacopuolos, M.. INSERM U 394, Bordeaux, France
The composition of interstitial fluid in bee retina: implications for the supply of substrates of energy metabolism to neurons

42. Cesar, M., and Hamprecht, B., Physiologisch-Chemisches Institut, Universität Tübingen, Germany
Immunocytochemical examination of neural primary cultures and brain slices using antibodies raised against pyruvate carboxylase

43. Chan, B., Garey, L. J., and Jen, L. S., Department of Anatomy, Charing Cross & Westminster Medical School, London, UK
Retrograde degeneration differentially affects the phagocytic and immunological functions of resident microglia in the rat optic nerve

44. Chapron, Jacqueline, de la Porte, Sabine, and Koenig, Jeanine, Neurobiologie Cellulaire, Université de Bordeaux, France
Post-natal maturation of the neuromuscular junction by Schwann cells

45. Chen, S. T., Wang, J. P., Garey, L. J., and Jen, L. S., Department of Anatomy, Charing Cross & Westminster Medical School, London, UK
ß-amyloid precursor protein immunoreactivity in normally developing and axotomised rat retinas

46. Chronwall, B. M. and Gary, K. A., Cell Biology and Biophysics, University of Missouri, USA
Transitions in protein expression by rat pituitary intermediate lobe glial-like cells during altered physiological states and development

47. Chvátal, A., Pastor, A., Sykova, E., and Kettenmann, H., Laboratory of Cellular Neurophysiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
Ion channel properties and morphology of glial cells in the developing rat spinal cord slice

48. Ciccarelli, R., Ambrosini, G., Di Iorio, P., Ballerini, P., Giuliani, P, Tiboni, G. M., and Caciagli, F., Inst. of Pharmacology, School of Med. University of Chieti, Italy
Changes of cultured astrocyte proliferation rate induced by ATP and related analogues

49. Ciesielski-Treska, J., Ulrich, G., Chasserot-Golaz, S., and Aunis, D., INSERM U 388, Center of Neurochemistry, Strasbourg, France
Protein tyrosine kinase c-YES associates with vimentin in cultured microglia

50. Coles, Jonathan A., INSERM U176, Université de Bordeaux, France
Analysis of the extracellular fluid in bee retina: implications for the supply of substrates of energy metabolism from blood to neurons

51. Collini, P., Armellini, R., Menhini, A. R., and Bocchini, V., Department of Clinical Medicine, Pathology and Pharmacology, University of Perugia, Italy
Interplay between genotype and phenotype in GL22 glioblastoma cell line

52. Colombo, J. A., Napp, M., and Puissant, V., Programa Unidad de Neurobiología Aplicada (PRUNA) (CEMIC-CONICET), Buenos Aires, Argentina
Radial glia: is it regulated by astroglial autocrinia?

53. Colombo, J. A., and Puissant, V., Programa Unidad de Neurobiología Aplicada (PRUNA) (CEMIC-CONICET), Buenos-Aires, Argentina
Transitional glia and nonhomogeneities in cortical GFAP$^+$ astroglia in adult cebus apella monkeys

54. Copin, J. C., Pinteaux, E., Ledig, M., and Tholey, G., Centre de Neurochimie du CNRS Strasbourg, France
Modulation of the astrocytic response to a reactive oxygen species generating environment

55. Däubner, W., Wernet, P., Seghrouchni, S., Remscheid, C., Fischer, H. G., and Hadding, U., Institut für Med. Mikrobiologie und Virologie, Universität Düsseldorf, Germany
Accessory cell function of glioblastoma U373MG

56. Deckert-Schlüter, M., Schlüter, D., Hof, H., Wiestler, O. D., and Lassmann, H., Institut für Neuropathologie, Universität Bonn, Germany
Expression of cell adheson molecules and MHC class I and II antigens in the normal and inflamed choroid plexus and ependyma

57. Deitmer, Joachim W., and Schneider, H. P., Abteilung für Allgemeine Zoologie, Universität Kaiserslautern, Germany
Membrane potential-dependent clamp of intracellular pH in identified leech glial cells

58. Del Bigio, M. R., Deck, J. H. N. and Davidson, G. S, Division of Neuropathology, University of Toronto
Glial cells in human brains ingest serum proteins following hemorrhage

59. Deloulme, J. C., Lucas, M., Keller, A., and Sensenbrenner, M., LNO-Centre de Neurochimie du CNRS, Strasbourg, France
Expression and regulation of α and γ enolase subunits during the differentiation of rat 0-2A progenitor cells into oligodendrocytes

60. tom Dieck, S., Schulz, M., and Bauer, K., Max-Planck-Institute for Experimental Endocrinology, Hannover, Germany
Uptake of dipeptide derivates by astroglia rich rat brain primary cultures

61. Distler, C., Dreher, Z, and Hoffmann, K.- P., Allgemeine Zoologie und Neurobiologie, Ruhr-Universität Bochum, Germany
Müller cells in the primate retina

62. Dolbois, L., Coppin, H., Borot, N., Yassine-Diab, B., Pontarotti, P., Clanet, M., and Roth, M. P., CNRS UPR 8291, Toulouse, France
DNA polymorphisms at the human myelin oligodendrocyte glycoprotein (MOG) locus.

63. Donato, R., Bianchi, R., Garbuglia, M., Falleri, S., and Verzini, M., Section of Anatomy, University of Perugia, Italy
S-100 protein and calpactin 1 bind different sites on GFAP and differently affect GFAP assembly

64. Dong, Z., Jessen, K. R., and Mirsky, R., Department of Anatomy and Developmental Biology, University College London, UK
Age-related Schwann cell have different mitogenic response to mitogens

65. Dubovy, P., Svizenska, I., and Bednarova J.. Department of Anatomy, Medical Faculty, Brno, Czech Republic
The basal lamina can stimulate a differentiation of migrated Schwann cells in the peripheral nervous system of the adult rat

66. Eclancher, F., Labourdette, G., and Sensenbrenner, M., LNO - Centre de Neurochimie du CNRS Strasbourg, France
Astrogliosis following bFGF, PDGF or EGF microinjection in the intact or injured brain of adult rat

67. Eder, C., Fischer, H.-G., and Heinemann, U., Institute of Neurophysiology, University of Cologne, Germany
Properties of potassium currents of microglial cells under different culture conditions

68. Eins, S., Rohlmann, A., and Wolff, J. R., Department of Anatomy, University of Göttingen, Germany
Characterization of astrocytic lamellae by morphometrical and fractal parameters

69. Engel., Jutta, Althaus, H. H., and Kristjansson, G. I., Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany
NGF increases $[Ca^{2+}]_i$ in regenerating mature oligodendroglial cells

70. Evrard, M. - E., Di Scala-Guenot, D., and Strosser, M. T., Institut de Physiologie, URA 1446, Strasbourg, France
Oxytocin-binding sites in rat spinal cord cultured astrocytes

71. Faff, L., Reichenbach, A., Dettmer, D., and Albrecht, J., Medical Research Centre, Polish Academy of Science, Warsaw, Poland
Comparative characteristics of NH_4^+ and K^+-induced [H]taurine (TAU) release from cultured Müller glia

72. Fages, C., Rolland, B., Leprince, G., Riol, H., Lefrancois, T., Arcouri, C., and Tardy, M., INSERM U282, Hôpital Henri Mondor, Creteil, France
GFAP gene expression during postnatal development

73. Fages, Christiane, INSERM U282, Hôpital Henri Mondor, Creteil, France
Regulation of the glial fibrillary acidic protein (GFAP) and of its encoding message in the developing brain and in cultured astrocytes. Modulation by various signals

74. Faissner, A., Clement, A., Lochter, A., Streit, A., Mandl, C., and Schachner, M., Department of Neurobiology, University of Heidelberg, Germany
Purification of a neural proteoglycan with neurite outgrowth promoting properties

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Demonstration of perineuronal nets in the auditory cortex of gerbills using confocal laser scanning microscopy

Synergie cérébrale



Duxil

Almitrine + Raubasine

L'alliance fondamentale

Déficit intellectuel du sujet âgé
et ischémie neurosensorielle

- Mémoire
- Vertiges
- Acuité visuelle
- Suites d'AVC

Duxil, 1 à 2 comprimés ou 40 à 80 gouttes par jour

Formes, présentations et composition : Boîte de 30 comprimés enrobés - AMM N° 322 269 B - Prix : 70,40 F - Composition : dose par comprimé : Almitrine bimésilate 30 mg - Raubasine 10 mg ; dose par conditionnement : Almitrine bimésilate 900 mg - Raubasine 300 mg. Suspension buvable - flacon de 48 ml - AMM N° 322 295 5 - Prix : 70,40 F - Composition : dose pour 40 gouttes : Almitrine 30 mg - Raubasine 10 mg ; dose par conditionnement : Almitrine 900 mg - Raubasine 300 mg. Liste II - Remboursé S.S. (65 %). Admis aux collectivités. **Propriétés pharmacologiques essentielles :** Duxil enrichit le sang artériel en oxygène, répondant ainsi à l'hypoxie. L'activité de Duxil a été démontrée aussi bien avec des modèles animaux qu'à l'aide d'études de pharmacologie clinique chez l'homme. L'action de Duxil sur la PaO_2, la pression tissulaire d'oxygène (PO_2), l'utilisation cérébrale d'oxygène et l'électro-encéphalogramme est due essentiellement à l'almitrine, principe actif de Duxil, dont l'activité est potentialisée par la raubasine. **Indications thérapeutiques :** • Proposé comme traitement correcteur des symptômes du déficit intellectuel pathologique du sujet âgé (troubles de l'attention, mémoire...). • Proposé dans : – les troubles sensoriels d'origine ischémique, – les troubles cochléovestibulaires d'origine ischémique. • Utilisé dans les suites immédiates et tardives des accidents ischémiques cérébraux. **Contre-indications :** Ne pas associer aux I.M.A.O. **Précautions d'emploi :** Au niveau neurologique, l'apparition et la persistance de fourmillements, picotements ou engourdissements au niveau des membres inférieurs amènent qu'une perte de poids supérieure à 5 % doivent faire interrompre le traitement. Ces signes apparaissent lors de traitements de longue durée et régressent à l'arrêt du traitement. La survenue d'une neuropathie périphérique reste très rare : l'incidence apparente est de 5 cas pour 100 000 mois-malade, elle doit faire interrompre le traitement. Duxil ne doit pas être associé à d'autres préparations contenant de l'almitrine (Vectarion). Ne pas dépasser la posologie préconisée. Chez l'hypertendu, le traitement par Duxil ne dispense en aucun cas d'un traitement spécifique de l'hypertension artérielle. Pour la suspension : en cas d'administration aux diabétiques, tenir compte dans la ration journalière de la quantité de sucre comprise dans les excipients (40 gouttes = 0,48 g de saccharose). Grossesse : l'usage de Duxil est déconseillé chez la femme en période d'activité génitale et particulièrement pendant les premiers mois de la grossesse à moins que, de l'avis du médecin, l'utilité thérapeutique ne l'emporte sur les risques éventuels. **Effets indésirables :** Il a été signalé de rares effets secondaires : – amaigrissement, neuropathies périphériques avec sensations anormales au niveau des membres inférieurs ou paresthésies (picotements, fourmillements, engourdissements...). Ces effets ont généralement été constatés au cours de traitements à long terme, 1 an et plus. Ils conduisent à interrompre le traitement (cf. Précautions d'emploi) ; – nausées, brûlures et pesanteur épigastriques, troubles dyspeptiques, troubles du transit, troubles du sommeil à type d'insomnie, somnolence, agitation, anxiété, palpitations, sensations vertigineuses. **Mode d'emploi et posologie :** 1 à 2 comprimés ou 40 à 80 gouttes par jour en 2 prises régulièrement espacées. 40 gouttes = 1 comprimé. Coût journalier : 2,34 à 4,68 F. **Durée de conservation :** 2 ans. Pour une information plus complète, se reporter au dictionnaire des spécialités. Les Laboratoires Servier, 45520 Gidy. **TSERVAL** (Dist.) 27, rue du Port – 92200 Neuilly-sur-Seine. Tél. : 46.41.60.00.

1. • élève la pression en oxygène du sang artériel et du tissu cérébral
   • assure un bon apport en substrat oxydatif, accroît l'action métabolique de la raubasine
2. • réduit les résistances vasculaires par action centrale sur les récepteurs α 1 et α 2 (Roquebert, Arch Int Pharmacodyn 1988 ; 292 : 252-61)
   • améliore le métabolisme énergétique neuronal, potentialise l'action de l'almitrine

94 DU 07 PT

NOTES

Jeder kann in 6 Stunden blau sein .....



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CONTROL OF CELL NUMBERS BY NEUROTROPHINS

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During normal development of the vertebrate nervous system, many cells initially generated are eliminated when long lasting relationships are first established, for example when neurons contact their targets, or oligodendrocytes their axons. It is becoming apparent that cell numbers can be regulated by the limited availability of well defined molecules, some of which belonging to a gene family -the neurotrophins. There is experimental evidence to suggest that nerve growth factor (NGF) regulates cell numbers by preventing the death of sympathetic and neural crest-derived sensory neurons, and that brain-derived neurotrophic factor (BDNF) saves motoneurons both during development and after axotomy. However, recent results indicate that neurotrophin-3 (NT-3) regulates neuronal numbers in vivo long before normally occurring cell death is seen in NT-3-responsive ganglia. Already during gangliogenesis, the limited availability of NT-3 regulates neuronal proliferation and/or the differentiation of neuronal progenitor cells. In addition, a direct mitogenic effect of NT-3 has been demonstrated by Barres, Raff and colleagues on oligodendrocyte precursor cells. It is thus becoming apparent that neurotrophins control cell numbers in the developing nervous system of more than one cell type by more than one mechanism.

MIGRATION OF MYELIN FORMING CELLS IN THE ADULT DEMYELINATED SPINAL CORD.

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In the demyelinated CNS, oligodendrocytes and their precursors, but also Schwann cells, the myelin forming cells of the PNS may participate to myelin repair. Their contribution to this phenomenom results either from proliferation and/or migration in response to signals present in the demyelinated lesions. To understand if and how oligodendrocyte precursors and Schwann cells are able to migrate within the adult CNS, both cell types were transplanted at a distance from a myelin lesion induced by lysolecithin in the mouse spinal cord and were found within the lesion which they were able to repair (Gout et al., Neurosci. Lett., 89, 195-199, 1988; Baron-Van Evercooren et al., J. Neuroimmunol., 40, 235-242, 1992). While these data suggested migration and attraction of these cells by the lesion, their modalities of migration in the adult CNS remained unexplored. Using the Hoechst dye to trace the transplanted cells in the host spinal cord at the optical level, several pathways of migration were identified. These include: the meninges, the margins of blood vessels, the ependymal wall and in certain conditions the white matter fasciculi (Baron-Van Evercooren et al., J. Neurosci. Res., 35, 428-438, 1993; Vignais et al., Int. J. Dev. Neurosci., 11, 5, 603-612, 1993). Using another fluorochrome, carbocyanine (DiI) which can be photoconverted and visualized at the ultrastructural level (Gansmuller et al., Neurosci. Lett.147, 151-154, 1992), transplanted cells were found in the same locations than observed at the optical level. In the meninges, cells were localized among the various layers of extracellular material and meningeal cells. Around blood vessels, cells were confined to perivascular spaces between endothelial and astroglial basement membranes. Along the ependyma, cells were found at the basal pole or between ependymocytes which are devoid of basement membrane. These data thus suggest that myelin forming cells migrate on various substrates and may use different mechanisms for their migration in the adult CNS. The role of cell surface molecules mediating cell-cell or cell-extracellular matrix interactions will be discussed.

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Periaxin, A Novel Cytoskeletal Protein Of Myelinating Schwann Cells

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We have identified a novel protein of rat peripheral nervous system (PNS) myelin. We have shown that this protein is primarily localized in the periaxonal membranes of myelinating Schwann cells, therefore we call it periaxin. Periaxin is encoded by a single-copy gene, although two mRNA species were detectable by Northern blotting. A highly basic domain at the N-terminus of the protein is separated from an acidic region at the C-terminus by a repetitive sequence that comprises a conserved pentameric sequence with a tripeptide (LPK) spacer. Periaxin appears to participate in the earliest events of myelination, since it is readily detectable by immunogold EM in the loose uncompacted whorls of PNS myelin that first ensheathe the axon. In the mature myelin sheath periaxin has a restricted distribution in that it is excluded from compact myelin, but is concentrated in those periaxonal regions of myelin that are filled with Schwann cell cytoplasm, where it colocalizes with the myelin-associated glycoprotein (MAG). Unlike MAG, periaxin is not enriched in Schmidt-Lanterman incisures or in paranodal loops. Periaxin remains completely insoluble after Triton X-100 extraction of both myelin preparations and intact Schwann cells, and it is phosphorylated in cytoskeleton-enriched fractions of PNS myelin. These are the properties that would be predicted for a cytoplasmic protein that associates strongly with the Schwann cell cytoskeleton.

Multiple phosphorylation cascades in astrocytes triggered by neurotransmitters and growth factors: Studies using PEA-15, a novel major astrocytic phosphoprotein, as a probe.

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Astrocytes express receptors through which these cells can respond to neuronal signals. Activation of these receptors induces a cascade of events, such as stimulation of protein kinases and subsequent phosphorylation of target proteins. For example, activation of PKA by cAMP and of PKC by calcium and diacylglycerol have been reported to induce morphological changes of astrocytes and phosphorylation of cytoskeletal proteins such as vimentin and GFAP, astrocytic intermediate filament proteins, and MARCKS, an actin cross-linking protein. To understand regulatory processes underlying neuron-glial interactions, attempts were made to identify major phosphorylated proteins in astrocytes. Two dimensional gel electrophoresis analysis of $^{32}PO_4^{3-}$-labeled cultured mouse astrocyte homogenates revealed a highly labeled doublet, Pa and Pb, with a low molecular weight (15kD) and acidic pI (5.2 and 5.3, respectively). Partial microsequencing of the doublet indicated that it represents two isoelectric variants of a single protein. Western blot analysis using antibodies raised against internal sequences confirmed this result and revealed in addition the nonphosphorylated form, N. This novel protein was named PEA-15, for phosphoprotein enriched in astrocytes-15kDa. Pa which most likely is phosphorylated on one site, exists under two forms Pa1 and Pa2. Analysis of phosphorylation sites within PEA-15, simultaneously performed in vitro and in intact cells, demonstrated that PEA-15 is a substrate for PKC and that the kinase mediates the transition from N to Pa2, and from Pa1 to Pb. The seryl residue phosphorylated in Pa1 is within consensus sequences for casein kinase II (CKII) and calcium/calmodulin kinase II (CaMK II). Interestingly, insulin and EGF which are known to stimulate CKII activity increased Pa1 phosphorylation. In addition, noradrenaline which raises intracellular calcium concentrations, also promoted Pa1 phosphorylation, suggesting that the seryl residue can also be phosphorylated by CaMK II.

TEMPORAL AND SPATIAL ASPECTS OF CALCIUM SIGNALING IN CULTURED RAT HIPPOCAMPAL ASTROCYTES

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Within the last few years, glial Ca^{2+} control has become a subject of intense study. This talk will review developments in the field and will examine aspects of glial Ca^{2+} oscillations and Ca^{2+} waves in more detail. Glial calcium levels respond to the activity of a wide variety of receptors and ion channels that are sensitive to neurotransmitters, neuropeptides, hormones and other small molecules. The Ca^{2+} responses range in complexity from simple monophasic transient increases to biphasic and polyphasic responses. Ca^{2+} responses may develop uniformly within a cell or propagate in the form of a wave. Most current models suggest that the same basic biochemical mechanism generates either Ca^{2+} oscillations or waves: the latter produced by considering the effects of diffusion. Results from analyzing the rate of signal change in various glial Ca^{2+} response types may offer insight into glial Ca^{2+} control as well as the mechanisms which shape glial Ca^{2+} transients.

A POSSIBLE LINK BETWEEN CELL ADHESION RECEPTORS, HOMEODOMAIN TRANSCRIPTION FACTORS AND NEURONAL IDENTITY.

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The orderly arrangement of neuronal cell bodies and axonal projections generated during nervous system development requires precise spatio-temporal control of the expression and activity of cell adhesion receptors. Recent evidence suggests that homeobox genes, an important class of developmental control genes, many of which are preferentially expressed in developping nervous tissue, play prominent roles in the regulation of expression of these molecules. We have characterised two mouse homeobox genes, named Cux and Phox2, the products of which bind to and regulate the promoter of the Ncam gene in vitro. Its highly specific expression pattern suggests that Phox2, besides regulating Ncam, may be a determinant of neuronal identity in the central and peripheral nervous system. Homeobox genes seem also to be involved in the differentiation of glial cells.

PLATELET FUSION TO ENDOTHELIAL CELLS: A CRITICAL MECHANISM OF BLOOD-BRAIN BARRIER CHANGES INDUCED BY TNF-α

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In vivo alterations of the blood-brain barrier were analyzed in an experimental model of cerebral malaria (CM). This complication of the acute phase of infection stems from injury to the brain microcirculation; it alone is responsible for the death of more than 2 million people annually. The neurovascular injury, leading to perivascular micro-hemorrhages, is associated with the sequestration of parasitized erythrocytes and macrophages in cerebral venules. In this model where TNF-α has been shown to have a central role, we demonstrate that platelets are the critical effectors of the neurovascular injury. First, by electron microscopy, we found that during CM platelets adhere and fuse to brain endothelial cells. Second, radiolabelled platelet distribution studies indicated the platelets sequester in the brain vasculature when CM develops. Non-cerebral malaria was not associated with cerebral sequestration of platelets. Third, treatment *in vivo* with anti- LFA-1 monoclonal antibody (mAb) selectively abrogated the cerebral sequestration of platelets, and prevented the mortality due to the neurological syndrome. The α (CD 11a) and, to a lesser extent, β (CD 18) chain of the integrin LFA-1 were found to be expressed on platelet membranes. Fourth, malaria-infected animals rendered thrombocytopenic were significantly protected against CM. Thus, a CD11a-dependent interaction between platelets and endothelial cells appears pivotal to microvascular damage.
Using cultures of mouse brain microvascular endothelial cells (MVEC), we investigated the adhesion molecules involved in platelet/endothelial fusion and some functional consequences of this phenomenon, specifically cytoadherence. We found that TNF-α induced adhesion of radiolabelled platelets to MVEC in a dose-dependent manner. This effect was amplified by IFN-γ. Conversely, mAbs to ICAM-1 or to its ligand, LFA-1, completely blocked the adhesion and fusion of platelets induced by these cytokines, confirming that fusion depends upon the β-2 integrin expressed on platelet surface. Electron microscopial and flow cytometric analyses showed that, as a result of fusion, platelet markers, such as LFA-1 or the platelet antigen recognized by our monoclonal anti-platelet antibody, CV5-H7, were readily transferred onto endothelial cell membranes. Binding studies indicated that fusion of platelets into MVEC significantly increased adherence for blood leukocytes. Taken together, our results indicate that fusion of platelets into MVEC represents an important phenomenon in the modulation of TNF-α effects in brain microvascular pathology. These data suggest a novel mechanism of action for anti LFA-1 mAb*in vivo* and illustrate an unexpected role of platelets in neurovascular pathology.

ON THE FUNCTION OF ASTROCYTE GLYCOGEN

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Studies were undertaken to help elucidating the function of brain glycogen. In adult rat brain it is the astrocytes, the ependymal cells and neurons of the mesencephalic sensory nucleus that contain the main enzyme of glycogen breakdown, glycogen phosphorylase. In astroglia-rich cultures derived from newborn rat brain glycogen is degraded by increasing the cytosolic concentration of Ca^{2+} or cyclic AMP, or by withdrawal of glucose from the culture medium. Astroglial cells deprived of an external supply of glucose release large amounts of lactic acid but no glucose. In contrast, cultured hepatocytes, used as positive controls, under such conditions do release their glucosyl residues of glycogen as glucose. If this finding were a model for astrocyte behavior in brain, it would mean that astrocyte glycogen could be considered a store of lactic acid rather than of glucose. As a valuable substrate for oxidative generation of energy, lactic acid may be a fuel material provided, on signalled "request", to support neighbouring cells such as neurons and oligodendrocytes. Compatible with this notion is the presence of transport facilities for lactic acid in both astroglia-rich and neuron-rich primary cultures.

SCHWANN CELL PRECURSORS IN THE RAT: SURVIVAL AND DEVELOPMENT

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The generation of Schwann cells from the neural crest is poorly understood. We show here that this takes place via the generation of a distinct intermediate cell, the Schwann cell precursor.

These cells are found in embryo day (E) 14/15 limb nerves. They have a flattened morphology in culture, do not express S100 and die abruptly between 5h and 10h after plating by apoptosis unless they are rescued by survival factors. Contact with neurites, or bFGF + IGF-1 prevents precursor death. In vivo, Schwann cell precursors generate Schwann cells (i.e. $S100^+$ cells with elongated morphology that survive without bFGF + IGF-1 or neuronal contact) during one brief period between E15 and E17.

MICROGLIA, THE FIRST LINE OF DEFENCE IN THE CNS

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Transition of microglial cells from a quiescent or resting state to an activated state occurs within hours after injury. MUC 102, an antibody recognizing microglia and brain macrophages, is a very sensitive marker of this activation, as is the amyloid precursor protein L-APP and the complement receptor CR3bi (Ox 42). Activation also leads to immune competence and the expression of the MHC class I & II molecules in microglia. Mitotic activity is seen after day 3 in microglial cells only and not in astroglia. Astrocytes, however, show hypertrophy and fiber formation (GFAP). They could be the source of colony stimulating factors (CSF) acting as mitogens for microglia which increasingly express CSF receptors. Cytokines such as IL-6 and TGF-beta have also been demonstrated to increase in reactive glial cells during the axotomy response in the facial nucleus.

The repertoire of regulation of significant immuno-molecules in activated microglia is not specifically related to a certain neuropathology. In contrast, regulation seems to be essentially the same in all cases of microglial activation, whether occurring in trauma, ischemia, inflammation, autoimmune diseases, intoxication or even cortical spreading depression. The microglia seems to act as a multi-purpose defence cell of the parenchyma. It is activated quickly and augmented by mitosis, it is an antigen presenting cell, has cytotoxic potential, is mobile and responsive to intercellular communication molecules and to changes in the ionic milieu and finally can become a brain macrophage. (cf. Special Issue: Microglia; GLIA 7:1-118, 1993).

OLIGODENDROCYTES IN MULTIPLE SCLEROSIS

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Although the pathology of human inflammatory demyelinating diseases such as multiple sclerosis has already been described extensively a century ago, very little is still known about the fate of oligodendrocytes. Recently new markers and techniques became available, which allow a detailed study of these cells in situ. Myelin oligodendroglia glycoprotein is a myelin antigen, that appears on the surface of oligodendrocytes very late during myelination. There it persists on preserved oligodendrocytes after demyelination. Oligodendrocytes, that are engaged in synthesis and maintenance of myelin can be selectively labeled by in situ hybridization using specific probes for proteolipid mRNA. Degenerating oligodendrocytes can be identified by combining these above mentioned techniques with in situ nick translation, that labels nuclei with DNA fragmentation. Using these techniques on multiple sclerosis tissue we found three essentially different patterns. In classical chronic MS oligodendrocytes are destroyed in active lesions, followed by only limited recruitment of new oligodendrocytes from progenitor cells. However, in MS lesions that occur during the first year of disease oligodendrocytes are largely preserved and remyelination is extensive. Finally in acute MS extremely severe inflammation leads to destructive lesions. Our data indicate that the pathogenesis of MS lesions may be different at different stages of the disease process.

DIFFERENTIATION OF GLIAL CELLS IN THE PERIPHERAL NERVOUS SYSTEM STUDIED IN THE AVIAN EMBRYO

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The glial cells of the peripheral nervous system (PNS) originate from the neural crest. Clonal analysis of the differentiation potentialities of neural crest cells have shown that the peripheral glial and neuronal cells originate from common pluripotent neural crest progenitors. We have been interested in the problem of the segregation of the glial and neuronal lineages during the ontogeny of the neural crest. For this purpose, we have looked for specific glial molecular markers which are expressed at an early stage of glial cell differentiation. Two such markers have been identified by monoclonal antibodies, 4B3 and SMP (for Schwann cell Myelin Protein). The former is a glycosylated epitope carried by glycolipids and glycoproteins. The latter is a glycoprotein of the Ig-like superfamily. The expression of these markers *in vivo* and in various culture conditions revealed that the SMP gene is regulated in glial cells by environmental cues. A model for the segregation of the glial lineage and for the differentiation of the various PNS glial cell subtypes is proposed on the basis of the expression of these and other specific glial cell antigens in the course of normal development and in experimental conditions.

NEUROTOXIC AND NEURITE PROMOTING EFFECTS OF BRAIN MACROPHAGES

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Brain macrophages (BM) also called amoeboid microglia occur physiologically in the developing CNS. These cells can also appear in the adult brain in different contexts of lesion.

In vitro cultures of cells derived from embryonic rat brain were used to study the effects of BM on neuronal survival and differentiation. We observed that BM can promote the death of cultured neurons as well as neurite growth or regeneration. Neuronal death was observed as a possible consequence of membrane contacts between BM and neurons and involved the release of oxygen free radicals by BM. Neurite growth was stimulated by BM secretion of thrombospondin, an extracellular matrix protein.

The regulation of these BM activities and their role in the formation or remodelling of neuronal networks will be discussed.

RECEPTOR-MEDIATED CHANGES IN ASTROGLIAL CALCIUM LEVELS: INTEGRATION OF SIGNALS AND "ACTION POTENTIAL LIKE" CALCIUM RESPONSES

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Individual astrocytes *in vivo* are likely to be exposed to multiple neuroligands simultaneously in a manner similar to neurons. In the case of neurons, excitatory and inhibitory signals are generally integrated and threshold levels of depolarization required to elicit propagated responses. Experiments were carried out to examine the relationship between the density of astroglial neuroligand receptors and the ability of these cells to respond with increases in intracellular calcium ($[Ca+i^{++}]$). The density of alpha$_1$-adrenergic receptors (α_1-ARs) on individual astroglia was quantified via receptor autoradiography following assessing calcium responses using a video-based imaging system. While the density of α_1-ARs correlated well with the probability of astroglia responding to phenylephrine (an α_1-AR selective ligand) with an increase in $[Ca_i^{++}]$, the magnitude of the calcium response was nearly independent of the density of α_1-ARs. There was a strong correlation between the density of α_1-ARs and the latency to response following neuroligand application. A robust increase in $[Ca_i^{++}]$ occurred when astroglia were exposed to two partial agonists that alone failed to increase $[Ca_i^{++}]$ indicating that astroglia integrate signals linked to calcium mobilization. Experiments with caged IP_3 and caged calcium suggest that IP_3 must build to a threshold concentration before stimulating an all-or-nothing calcium response in astroglia. Overall, our findings suggest that the regulation of calcium in astroglia resembles the regulation of action potentials in neurons. That is, ligands induce an all-or-nothing calcium response which initiates from a given point and then propagates through these cells in a regenerative manner. As with the generation of neuronal action potentials, it is the integration of different incoming signals that determines whether astroglia respond and the latency to response. The striking similarity between astroglial calcium responses and neuronal action potentials suggest that astroglial calcium signals may mediate intercellular signaling between these cells in brain.

NEUROPEPTIDES AS REGULATORS OF PROTEASE NEXIN-1 EXPRESSION IN CULTURED SCHWANN CELLS

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Cultured glial cells release a potent serine protease inhibitor with neurite promoting activity. Following the determination of the amino acid sequence, this 43 kD protein was named glia-derived nexin (GDN). It was subsequently shown that GDN and protease nexin 1 (PN-1), a thrombin inhibitor released by cultured human skin fibroblasts, are identical. In addition to thrombin, PN-1 also inhibits trypsin and the plasminogen activators u-PA and t-PA. As recent results demonstrate the presence of the inhibitor in a variety of cells, including neurons, the more general term protease nexin 1 (PN-1) should be used from now on.

The octapeptide angiotensin II (Ang II) was found to cause the down-regulation of Protease Nexin-1 (PN-1) expression in cultured Schwann cells. Displacement of Ang II binding using the selective receptor ligands losartan and CGP 42112 led to a several-fold induction of PN-1 over basal levels. Moreover, detection of angiotensinogen- and renin-mRNA in these cultures suggests an endogenous production of Ang II. Our data also demonstrate the presence of AT_1 and AT_2 angiotensin receptor subtypes in cultured Schwann cells as well as in the rat sciatic nerve. Among potential regulatory agents tested on Schwann cell primary cultures (cytokines, growth factors, mitogens, neurotrophins and neuroactive peptides), only angiotensin II (Ang II), calcitonin gene-related peptide (CGRP) and vasoactive intestinal peptide (VIP) affected PN-1 synthesis. Our results indicate that neuropeptides can differentially regulate the extracellular proteolytic activity, providing new aspects of neuron-glia interactions in the intact tissue and following nerve injury.

DOMINANT-NEGATIVE ACTION OF MUTATIONS IN THE MYELIN PROTEOLIPID PROTEIN (PLP/DM-20) GENE

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Point mutations in the gene for proteolipid protein (PLP), the major integral membrane protein of CNS myelin, result in severe hypomyelination of neurological mouse mutants (*jimpy*, *rumpshaker*) and in human families with *Pelizaeus-Merzbacher Disease*. Unexplained, the majority of mutations in this X chromosome-linked gene cause a marked lack of terminal glial differentiation and apoptotic cell death of most oligodendrocytes. The underlying cellular mechanism remains obscure, but it has been suggested that high-level expression of the misfolded membrane protein could be cytotoxic for oligodendrocytes. Heterozygous females are mosaics with respect to the X-linked PLP gene and it is not known whether the mutant allels are recessive at the cellular level. To circumvent experimentally the effect of X-chromosome inactivation and allelic exclusion, we have cloned the entire PLP wild-type gene and have reintroduced it into the mouse germ line. When autosomal PLP-transgenic mice were crossed into the available strains of PLP mutants, "heterozygous" mice could be generated all of which remained hypomyelinated. On closer inspection, however, the survival of oligodendrocytes was significantly enhanced. Thus, the mutant forms of PLP exert a dominant-negative effect, but apoptosis is not simply dictated by expression of misfolded proteins. We suggest that normal and mutant PLP polypeptides form oligomeric structures which are subject to protein degradation.

From glial precursors to glial tumours

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Our studies on the biology of oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells of the central nervous system will be discussed as a paradigm for studies on precursor cell biology and glioma cell biology. Specific topics addressed will be the control of precursor cell division and differentiation , the use of precursor transplantation in the repair of demyelinating damage, the biological differences between perinatal and adult precursor cells,the identification of growth factors which may be involved in promoting precursor cell self-renewal and remyelination in vivo, and the derivation and characterization of a human O-2A lineage glioma.

THE GENERATION OF CELLULAR DIVERSITY IN THE RAT CEREBRAL CORTEX

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In the cerebral cortex, as in other areas of the CNS, neurons and macroglial cells are derived from the precursor cells of the embryonic ventricular zone (VZ). The central question in neuroembryology is how this is achieved. Cell lineage studies *in vivo*, and studies of cortical cells in dissociated culture, indicate that during the period of corticogenesis, most VZ cells display only a limited developmental potential; most only generate one cell type, be it neuronal or glial, and the remainder seldom generate more that two cell types. Thus it seems that these VZ cells are specified to generate cells of a particular fate.

We have asked two questions regarding these specified VZ cells. First, we have sought to discover from which precursor cells they themselves are derived. Second, we have tried to discover what, in molecular terms, makes the different VZ cell types different. We have made a number of findings.

1. During the onset of corticogenesis, the neuroepithelium is composed predominantly of cells with quite different properties from the specified VZ cells that follow. In dissociated culture, these early cells divide symmetrically to give large clones, which fail to differentiate except to generate a few neurons or oligodendrocytes. Eventually, these cells die unlike the later specified precursor cells, which give rise to clones of differentiated neurons or glial cells even in serum-free culture media.
2. We have raised a number of monoclonal antibodies that appear to distinguish these different lineages. One in particular (3.19) recognises the early neuroepithelial cells, the specified neuronal precursor cells, and mature neurons, but does not recognise some precursor cells (we think those that generate glia), or mature astrocytes or oligodendrocytes. Our preliminary studies indicate that 3.19 recognises a phospho-peptide determinant shared by a number of proteins found in mature and immature neurons, but absent from glial lineages.

Controlling Oligodendrocyte Numbers

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We have studied the cell-cell interactions that control cell numbers in the oligodendrocyte cell lineage in the developing rat optic nerve. The final number of oligodendrocytes in the optic nerve depends on the number of precursor cells that migrate into the nerve, the number of divisions that the precursors undergo before differentiating, and the number of newly formed oligodendrocytes that undergo programmed cell death (PCD).

The number of precursor cell divisions depends on the intrinsic clock in the precursor cell that consists of at least two components: a counting mechanism that is driven by growth factors such as PDGF, NT-e and IGFs, and an effector mechanism that stops cell division and depends on extracellular hydrophobic signals such as thyroid hormone. The proliferation of the precursor cells in vivo depends on electrical activity in the axons in the nerve.

Fifty percent of newly formed oligodendrocytes die during normal development, apparently because they fail to get sufficient stimulation by extracellular survival factors that the cells seem to need to avoid PCD. PDGF, NT-3, IGF-1 and CNTF all support the survival of newly formed oligodendrocytes in vitro and suppress their death in vivo. Oligodendrocyte survival in the developing nerve depends on axons, but in this case electrical acitivity is not required.

We suspect that the cell number control operates in much the same way for many types of vertebrate cells, and that such cells are programmed to kill themselves unless they are continously signalled by other cells not to do so.

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CELL LINEAGE-SPECIFIC MALIGNANT TRANSFORMATION IN THE DEVELOPING NERVOUS SYSTEM OF THE RAT

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Oncogenesis - as induced by DNA-reactive carcinogens - is a multistep process that deranges proliferative controls and the differentiation program of cells, and culminates in the clonal development of tumorigenic phenotypes. Depending on both the type and developmental/differentiation stage of the target cells and the molecular reactivity of the respective carcinogenic agent, malignant transformation involves mutational and/or functional alterations of distinct genes whose expression is connected with critical steps in the differentiation pathways of particular cell lineages. In the rat, the overall neuro-oncogenic effect of a single exposure to the well-defined mutagenic N-nitroso carcinogen N-ethyl-N-nitrosourea (EtNU) thus varies with the "developmental window" chosen for the carcinogen-pulse, as do the relative proportions of different types of neural tumors arising in the peripheral (PNS) and central nervous system (CNS). Examples will be presented of specific genes whose mutation and/or altered expression is associated with EtNU-induced malignant conversion in specific cell lineages of the rat PNS and CNS; i. e. the *neu (erbB-2)* gene and its mutant counterpart involved in the development of malignant schwannomas, and a recently identified cell surface glycoprotein ($gp130^{RB13\text{-}6}$) associated with EtNU-induced neuro-oncogenesis in the CNS.

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GLIA AND THE BLOOD-BRAIN BARRIER

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The blood-brain barrier maintains brain homeostasis which is critical for neuronal function. In higher vertebrates, the barrier is formed by endothelial cells of brain capillaries. Complex tight junctions and a low density of pinocytotic vesicles are the structural basis for barrier function while specific transport systems carry essential nutrients and metabolites into the brain. A hallmark of brain capillaries is the presence of astrocytic endfeet which occupy the entire perivascular space and attach to the vascular basal lamina. There is evidence that astrocytes are responsible for inducing and maintaining blood-brain barrier properties but direct evidence is sparse and the signalling molecules involved in this process remain unknown. We have studied the development of the blood-brain barrier in avian and rodent embryos and have provided evidence that barrier properties are induced early after invasion of capillary sprouts into the neuroectoderm. In vitro, brain endothelial cells rapidly lost barrier properties which could be prevented at least partially by culturing them in medium conditioned by astrocytes. A synergistic effect on some properties was seen by elevating intraendothelial cAMP levels.

VOLTAGE-GATED K^+ CHANNELS IN RABBIT SCHWANN CELLS

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Rabbit Schwann cells generate outward K^+ currents when subject to a step depolarization from a hyperpolarized holding potential. Three types of voltage-gated channel contribute to these outward currents (types I, II & III) that can be clearly discriminated on the basis of their voltage-dependence, kinetics, pharmacology and single-channel characteristics (Baker et al., 1993; Howe et al., 1992; unpublished observations). The type I channel appears similar to the translation products of Kv 1.1 mammalian *Shaker* clones isolated from rat brain. The channel has a conductance of 12 pS and a linear current-membrane potential relation between -60 & +60 mV with 5.6 mM $[K^+]_o$, 170 mM $[K^+]_{in}$. Macroscopic type II current activates more slowly than type I current and the apparent threshold for type II current activation is about 20 mV more positive than that for type I, at close to -40 mV. Type II current inactivates with a time constant close to 2 s at 0 mV (20-22°C). The type II channel has a chord-conductance of 2.6 pS at 0 mV, and rectifies strongly in the outward direction (5.6 mM $[K^+]_o$, 170 mM $[K^+]_{in}$). Current generated by type III channels accounts for only a minor fraction of the total macroscopic K^+ current at positive membrane potentials in our experiments. The type III channel has a chord conductance of 58 pS at 0 mV and also rectifies outwardly with the same $[K^+]$ gradient. It appears most similar to the BK channel type. Pharmacological evidence strongly suggests that the rabbit type I channels and those contributing to macroscopic G_{Kf1} in amphibian axons differ -- perhaps reflecting amino-acid sequence differences.

THE ROLE OF OLIGODENDROCYTES FOR THE MATURATION, STABILIZATION AND REGENERATION OF THE CENTRAL NERVOUS SYSTEM

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Oligodendrocytes start to differentiate and form myelin when the axons of a given tract have reached their targets. Deletion of oligodendrocytes from rat optic nerves gave evidence for two roles of these cells in addition to myelin formation: the normal postnatal increase (5 - 20 x) of the axon diameters was completely prevented in the myelin-free parts of the axons, and sprouting occurred spontaneously along the fibers (Colello et al. and Colello and Schwab,, J. Neurosci., in press). In parallel, the normal, pronounced down-regulation of GAP-43 did not occur. In the spinal cord, a similar local effect of oligodendrocytes/myelin on the down-regulation of GAP-43 was observed, a fact which fits well with the distribution of GAP-43 in the adult, normal CNS where unmyelinated areas are much higher in GAP-43 than myelin-containing regions (Kapfhammer and Schwab, J. Comp. Neurol., and Europ. J. Neurosci., in press). Most of these areas that are low in myelin and high in GAP-43 are known to be among the most plastic parts of the CNS. Restriction or local prevention of sprouting and plasticity may, thus, be an important role played by oligodendrocytes. Candidate molecules for such a function are the neurite growth inhibitory proteins which are part of oligodendrocyte membranes and CNS myelin (Schwab et al., Annu. Rev. Neurosci. 16: 565-595, 1993). Neutralization of these proteins by antibodies leads to an increased plasticity of retinal fibers in the superior colliculus (Kapfhammer et al., J. Neurosci. 12: 2112-2119, 1992), and to long-distance regeneration of 5 - 10 % of the transected corticospinal tract fibers in the rat (Schnell and Schwab, Nature 343: 269-272, 1990, Europ. J. Neurosci. 5: 1156-1171, 1993).

THE MOLECULAR BASIS OF PERIPHERAL NEUROPATHIES IN MOUSE AND HUMAN

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Spontaneous mutations, including point mutations, DNA inversions, duplications or deletions, in the myelin genes underlie the disorders of myelin formation and maintenance in the PNS and CNS of animals and humans. Missense point mutations in PMP22, an intergral Schwann cell membrane glycoprotein (Welcher et al., 1991, PNAS USA, 88, 7195; Spreyer et al., 1991, EMBO J., 10, 3661), for example, are found in the mouse mutants *trembler* and *trembler-J*, characterized by severe myelin deficiency in the PNS, and in a small number of families with the corresponding Charcot-Marie-Tooth (CMT) disease type 1A. In the majority of CMT1A individuals the PMP22 gene maps within a large chromosomal duplication on chromosome 17. The reciprocal deletion of this region, including the PMP22 gene, is found in individuals with another neuropathy HNPP. Myelin formation appears to be critically dependent on the synthesis of the appropriate amount of PMP22 protein. Point mutations in myelin protein Po are found in CMT1B disease and in the gap junction protein connexin 32 in CMT1X disease. The PNS PMP22 and connexin 32 proteins like PLP in the CNS have four putative transmembrane domains suggesting similar possible roles in forming membrane pores. A characteristic feature of peripheral neuropathies besides myelin deficiency is continued Schwann cell proliferation raising the further possibility, emphasized also by the identity of the PMP22 and gas-3 genes, that PMP22 acts as a growth regulator. In this regard two alternative mRNA transcripts of the PMP22 gene have been identified, one expressed preferentially in neuronal and the other in non-neuronal tissues. The former is significantly regulated after nerve injury while the regulation of the latter is dependent on the growth state of the cell. Alternative splicing of the PMP22 may permit the PMP22 to function both as a structural myelin protein and as a growth regulator.

METABOLIC INTERACTIONS AND SIGNALS TRAFFICKING BETWEEN GLIAL CELLS AND NEURONS

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Among the variety of roles and diverse possible functions that have been attributed to glial cells, the nutritive function is strongly supported by direct experimental evidence obtained in the model-system of the honeybee drone retina. In this nervous tissue, with crystal-like structure, and in which glial cells and photoreceptor-neurons constitute two distinct metabolic compartments, glial cells transform glucose to alanine and, with proline, fuel the mitochondria of the photoreceptors. Proline supplies the Krebs cycle by making glutamate and, in turn, α-ketoglutarate. Light stimulation of the photoreceptors causes 200% increase of their oxygen consumption and 50% decrease of the pool size of proline and of glutamate. The use of proline meets a necessary anaplerotic function in the Krebs cycle but implies high NH_3 production in the photoreceptors. There is indeed a light-induced rise of ammonia in the extracellular space of the superfused retina measured with microelectrodes which is relatively small because NH_3 is fixed in the reactions of glutamine and alanine synthesis. Alanine formation fixes NH_3 at a rate far exceeding glutamine formation. This is consistent with the rise of a glial pool of alanine upon photostimulation. The finding showing a substential ^{15}N-alanine formation from ^{15}N-proline strongly suggest that ammonia and possibly glutamate are transferred from photopreceptor-neurons to glial cells. In the glial cells two predominantely cytosolic enzymes, qlutamate-dehydrogenase and alanine amino-transferase, work in tandem. The increase in NH_4 results in an increase in glutamate production which in turn activates the formation of alanine. The consequence of this activation by NH_4 is the accelaration of pyruvate turnover and an increase of NAD^+ available for glycolysis. Indeed, stimulation of photoreceptors induce an increase of glycolysis in the glia.

GROWTH FACTORS AND ASTROGLIAL CELLS: FOCUS ON FGFs, TGFßs AND CNTF

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FGFs and TGFßs are multifunctional cytokines with prominent and partially synergistic effects on cell growth and differentiation. FGF-1 and 2 and TGF-ß 1,2, and 3 are the most relevant members of the respective gene families with regard to the CNS: Their production and release by neurons, macro- and microglial cells suggest that they are important constituents of neural developmental, maintenance, and repair mechanisms. This talk will review several examples of their *in vitro* and *in vivo* biology in relation to astroglial cells including autocrine regulation, effects on cell division, proteases and protease inhibitors, cellular progenitors, transition into reactive astroglia, scar formation, and production of neurotrophic factors. CNTF is another pleiotropic cytokine produced by astrocytes. Regulation of its synthesis by cell density and exogenous CNTF will be reported. Finally, the role played by astrocytes and growth factors in models of Parkinson's disease will be addressed.

THE NITRIC OXIDE SYNTHASE - cGMP CONNECTION IN THE RAT BRAIN AND THE INVOLVEMENT OF GLIAL CELLS.

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The function of nitric oxide (NO) as an intercellular messenger in the brain was first suggested by Garthwaite (Garthwaite et al. Nature 336, 385 (1988) During the following years data accumulated which pointed to the nitric oxide synthase (NOS) - cGMP pathway as a (potentially) important transduction system. However, in the CNS the physiological significance of this system is still largely unknown.

Immunocytochemistry of cGMP can be used to study the localization and the metabolism of cGMP at a (sub)cellular level. The use of brain slices permits the manipulation of cGMP metabolism at various sites in the transduction cascade from activation of NOS to inhibition of selective phosphodiesterase(s). We demonstrated discrete populations of astrocytes which responded to a stimulus of ANF with an increase in the synthesis of cGMP. Furthermore we could provide visible evidence for the suggestion of Garthwaite that NO has an intercellular messenger function in the cerebellum of the rat. To further study the role of NOS in brain function we attempted to induce the inducible form of NOS in the rat brain by activation of (micro)glial cells using various protocols. Introduction of a push - pull lesion in the striatum of the rat resulted in reactive glial cells around the needle tract. However no evidence was found for the induction of NOS_i in any type of cell during the time period studied (6 - 12 - 18 - 24 - 48 hrs after lesion). Combination of this needle lesion with (pre)treatment with LPS, or concomitant implantation of fetal substantia nigra cells did not result in NOS_i induction in the intact rat brain. NO mediated cGMP synthesis was visualized in varicose fibers around the needle tract and in the cell implant but not in glial cells.

In conclusion, we found evidence for the involvement of glial cells in the functioning of the NOS - cGMP transduction pathway in discrete areas of the rat brain. The complexities of the regulation of cGMP synthesis and degradation in the CNS are only just beginning to be understood.

THE EFFECT OF CALMODULIN ANTAGONIST R24571 ON SWELLING-INDUCED K+ EFFLUX AND INFLUX IN C6 GLIOMA CELLS

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It is known that in cells from a number of mammalians Ca++ controls the volume-induced K+ efflux and reaction of regulatory volume decrease (RVD) (McCarty and O'Neil, Physiol. Rev., 1992, 72, 1037-1061). The placement of cells in a hypotonic (180 mOsm) calcium-free medium containing 0.5 mM EGTA does not prevent swelling-induced 86Rb+ efflux from isotope preloaded cells and is without significant influence on the activation of influx, mediated by sodium pump and Na+/K+/2Cl- cotransport. Preincubation of the cells with R24571 (10 μM) inhibits the swelling-induced stimulation of radiotracer efflux by 50-80%. At the same conditions there is no notable effect of the antagonist on the sodium pump activation. By contrast, the complete suppression of volume-stimulated Na+/K+/2Cl- cotransport is observed. It is suggested that swelling-induced 86Rb+ efflux through nonidentified K+ channels as well as the stimulation of inward-directed Na+/K+/2Cl- cotransport are calmodulin-dependent processes.

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REGULATION OF LEUKEMIA INHIBITORY FACTOR, PLATELET-DERIVED GROWTH FACTOR AND FIBROBLAST GROWTH FACTOR SYNTHESIS IN CULTURED HUMAN ASTROCYTES

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Elevated concentrations of several cytokines, including IFN-γ, TNF-α, IL-1β, IL-6 and TGF-β have been demonstrated in the cerebrospinal fluid and brain parenchima in association with inflammatory and degenerative disorders of the CNS. Using astrocyte-enriched cultures from the human embryonic brain, we have investigated the effects of the above cytokines on the synthesis and secretion of LIF, PDGF and FGF. Untreated astrocytes expressed LIF mRNA and secreted LIF protein. LIF synthesis was increased by the cytokines IL-1β, TNF-α and TGF-β_1, and by phorbol esters. The protein kinase inhibitors H7 and staurosporine partially antagonized cytokine-induced LIF release. Astrocytes synthesized and released LIF after subculturing, but not when growing in primary cultures in close contact with neurons. Using a PDGF AB specific ELISA, PDGF was detected in the supernatants of untreated astrocytes. PDGF levels were increased following treatment with TNF-α and TGF-β. We are currently investigating the effects of the above cytokines on the expression of PDGF A- and B-chain at the mRNA level. Neither untreated nor cytokine-treated astrocytes secreted basic FGF, as determined by specific ELISA; FGF immunoreactivity was present only in astrocyte lysates. The present findings suggest that induction of astrocyte LIF and PDGF secretion might occur in pathological conditions as a consequence of a local increase in the levels of inflammatory cytokines and/or alterations of neuronal-glial interactions.

OLIGODENDROCYTES ISOLATED FROM PIG OR RAT, ARE BOTH TARGET CELLS FOR GROWTH FACTORS AND CYTOKINES, BUT RESPOND TO A DIFFERENT SPECTRUM.

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Growth factors and/or cytokines play an important role for the differentiation, maturation, destruction and regeneration of oligodendroglial cells (OL). By testing various factors it was found that NGF can induce a subset of mature OL, isolated from pig brain, to proliferate and that NGF accelerates the OL process regeneration. These OL did not respond to growth factors (within reasonable conc.) which direct rat glial progenitors to proliferate or to become astrocytes (type 2) or OL. Rat OL did not respond to NGF, but may be NT-3 responsive (as published recently.) OL, isolated from the brain of adult baboons, behave similarly as pig OL when exposed to NGF.
Pig and rat OL appear also to react differently when exposed to TNF: Concentrations, which have been reported to destroy a substantial number of rat OL do not affect pig OL grown in culture under similar conditions.
These and other (molecular) data suggest that

1.) The response to individual growth factors or cytokines may be species dependent
2.) The set of receptors at the plasma membrane may vary during ontogenesis
3.) Pig OL may reflect the response program of human OL better than rat OL, which is important to note when considering growth factors and cytokines for eventual therapeutical use.

MITOSIS OF SCHWANN CELLS INDUCED *IN VIVO* BY APP AND OTHER PROTEASE INHIBITORS.

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The amyloid precursor protein with antiprotease activity (APP K+), aprotinin and leupeptin were locally supplied to rat peripheral nerves and studied under the electron microscope. At days 5-10 of exposure, but not at day 2, mitosis of Schwann cells were observed regularly. The associated axons were always normal, and also the myelin sheaths, although in some cases these were disrupted. Demyelinated fibres were found frequently; axons being extruded through a broken myelin sheath were rare. The nuclei of Schwann cells associated with demyelinated fibres were tenfold those of the cells whose myelin was normal; in a cross section, even two nuclei (cells) were associated with an axon. A few mm central and distal to the treated segment, the nerve was normal, i.e, the effect of protease inhibition is strictly local. Administration of vehicle, APP K+ heated before injection, or APP K-, produced no effect.

Our results indicate that an extracellular protease is instrumental to repress the cell cycle of Schwann cells in mature nerves, and that once they are committed to proliferate, resorb the myelin sheath. The mitogenic activity of APP may be involved in the pathogenesis of Alzheimer´s disease.

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MEMBRANE RESPONSES TO EXTERNALLY APPLIED ATP IN MOUSE SCHWANN CELLS FROM ORGANOTYPIC CULTURE OF DORSAL ROOT GANGLIA

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Within the nervous system, convincing evidence has been recently put forward that ATP acted as a fast excitatory synaptic transmitter and therefore it is tenable that extracellular ATP released by neurons could act on surrounding glial cells. In this study, we have investigated the effects of externally applied ATP on Schwann cells from organotypic culture of mouse dorsal root ganglia.

Experiments were performed using the whole-cell configuration of the patch-clamp technique at room temperature on Schwann cells that had been cultured for 1 to 8 weeks. We found that external application of ATP (10 µM to 10 mM) induced from a holding potential of -70 mV an inward current (I_{ATP}). ATP analogues were tested and gave the following relative agonist potency sequence: ATP=ATP-γ-S >ADP>>α-β-Metylene ATP. AMP and adenosine did not elicit any detectable membrane currents. Suramin, a putative P_2 purinoceptor antagonist reversibly reduced I_{ATP}. In order to work out the ionic nature of I_{ATP}, cationic and anionic gradiants were altered by ionic substitution. We found that ATP activated both a cationic conductance highly permeable to K ions and poorly permeable to Ca ions, and an anionic conductance with the following relative permeability sequence: SCN>I>Cl>aspartate>isethionate. Intracellular Ca did not seem involved in I_{ATP} as no differences were observed with or without 10 mM EGTA in the patch-pipette solution.

In summary external ATP by activating an inward current in mouse Schwann cells, triggers a membrane depolarization which could in turn activates voltage-dependent Ca channels and therefore modulates intracellular calcium concentration. If ATP is released by DRG neurons during electrical activity, it could act on surrounding Schwann cells and therefore could modulate electrical properties of mammalian Schwann cells.

EFFECTS OF PKC AND PKA STIMULATIONS ON CELL MORPHOLOGY, GFAP AND GS EXPRESSION IN A GLIOBLASTOMA MULTIFORM CELL LINE OF CLONAL ORIGIN.

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PKC and PKA activation by phorbol 12-myrystate 13-acetate (PMA) and dibutyryl cyclic AMP (dbcAMP) have been investigated in a glioblastoma multiform cell line (GL 15) showing an undifferentiated phenotype.
Glial fibrillary acidic protein (GFAP), and glutamine synthetase (GS) expression have been analyzed. PMA (100nM), induces both fast morphological changes, detectable after 5h of contact and an arrest of the proliferation. The GFAP-mRNA level reduces slowly after 3h followed by a dramatic decrease after 8h and 16h.,whereas the GFAP protein level.remained unchanged. PMA does not influence the GS mRNA and GS levels.
The GL-15 control cells, grown in serum-free medium, present a differentiated morphology associated with a high level of GFAP-mRNA and GFAP protein. When compared to GL 15 control cells, a dbcAMP concentration of 1mM induces a regression of the morphology to an undifferentiated form associated with a GFAP immunolabelling decrease. These results are confirmed by Northern-blots and Western-blots that show a time-dependent reduction of both,GFAP-mRNA and GFAP levels. Moreover dbcAMP determines a time-dependent reduction of GS-mRNA and GS protein level.
In normal astrocytes the PKA activation by dbcAMP induces biochemical and morphological differentiation, dbcAMP treated GL 15 cells show an opposite behavior. GL 15 cells respond to PKA activation with a process of biochemical and morphological regression.
In GL 15 cells, therefore, the two systems act throught different and opposite mechanisms. Alterations in the PKA signalling system appear evident. Further investigation will be necessary to find out the level of these anomalies and to investigate their role in the transformation mechanism.

CYTOSINE ARABINOSIDE INDUCES STRUCTURAL CHANGES IN DEVELOPING POSTNATAL RAT OPTIC NERVES

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It is becoming widely accepted that neurons and glia are involved in reciprocal communication, which represents a prerequisite for normal development of the nervous system. We are interested in establishing an experimental in situ model which allows the study of central nervous system development while the proliferation of glial cells is impaired. Cytosine arabinoside (Ara-C) was used as a S-phase specific antimitotic agent inhibiting DNA but not RNA synthesis of glia. The drug was administered intraperitoneally on four consecutive days starting with newborn animals. Volumes and concentrations of the administered Ara-C were determined experimentally. At postnatal day 5 the rats were sacrificed by intracardiac perfusion fixation and the following organs were removed for structural and immunocytochemical investigation : eye, optic nerve, brain, trigeminal nerve and skin. Here, the main structural changes within the optic nerves of the Ara-C treated animals are briefly described; the observed changes were absent in the control groups. The external glial limiting membrane showed irregularities in its course and extension, and was discontinuous at certain places. Capillaries were clearly reduced in number and the endothelial cells showed a cuboidal shape in contrast to their normal flattened appearance. A high ratio of glia to axon surface area was estimated on 1µm plastic sections and was interpreted as severe gliosis; the astrocytic cell processes appeared thicker and longer than in the controls. Electron microscopic and immunocytochemical investigations are in progress. Furthermore supplementary results obtained from additional experiments will show the usefulness of the proposed model system. Supported by MRC and ERC grants.

ADP-RIBOSYLATION PROCESS OF NUCLEAR PROTEINS IN GLIAL CELL CULTURES AND FETAL RAT BRAIN TISSUE SLICES TREATED WITH GROWTH FACTORS

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ADP-ribosylation is a post-translational modification of nuclear proteins and may play a crucial role in DNA repair, cell proliferation and differentiation. Epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and insulin-like growth factor I (IGF-I) stimulate astroglial and neuronal cell proliferation and differentiation in culture.

In the present research we studied the effect of growth factors (EGF, bFGF, IGF-I and insulin) on poly-ADP-ribose polymerase (pADPRP) activity, the key enzyme involved in nuclear protein poly-ADP-ribosylation, in purified nuclei from primary rat astroglial cell cultures or fetal brain tissue slices of 18-day-old rat embryos. A marked and significant increase of pADPRP activity in 12 h bFGF (+140 %) or IGF-I (+100 %) treated astroglial cell cultures was found; a lower, but considerable increment of the above mentioned nuclear enzyme in 2 h IGF-I or bFGF-treated fetal rat brain tissue slices was observed.

In further experiments the effect of different incubation times (1 or 2 h) and final concentrations of the growth factors (10 or 30 or 60 ng/ml for EGF, and 10 or 30 or 60 µg/ml for insulin) on pADPRP activity in EGF or insulin-treated fetal rat brain tissue slices was investigated. A significant stimulation of pADPPR activity in purified nuclei after 1 h treatment with EGF (60 ng/ml) and after 1 or 2 h treatment with insulin (60 µg/ml) was found. The addition of both insulin (60 µg/ml) and EGF (60 ng/ml) significantly increased nuclear protein ADP-ribosylation after 1 h, while the effect with lower concentrations of both insulin (10 µg/ml) and EGF (10 ng/ml) was observed after 2 h treatment.

In conclusion, our data show that these growth factors have a stimulatory effect on poly-ADP-ribosylation of nuclear proteins, and may play an important role in the regulation of gene expression in cultured astrocytes and fetal rat brain tissue slices.

TEMPORAL EXPRESSION OF β1 AND α1 THYROID RECEPTORS IN THE BIPOTENTIAL CELL LINE, CENTRAL GLIA-4 (CG-4)

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The importance of thyroid hormones in the differentiation of glial cells is well established and their effects are mediated by the presence of nuclear triiodothyronine (T3) receptors (T3Rs). Two genes coding for T3 binding receptors (T3Rα1 and T3Rβ1) and for a non T3 binding receptor (T3Rα2) have been isolated and found in the developing and the adult rat brain. The T3R isoforms expressed by 0-2A progenitors, oligodendrocytes (OL) and type-2 astrocytes are unknown. To determine which types of T3Rs are expressed during the differentiation of glial cells, we used a permanent cell line CG-4 of rat CNS progenitor cells O-2A, which are capable of differentiating into OL or type-2 astrocytes depending of the culture conditions (Louis et *al.*; J. Neurosci. Res. *31* : 193-204, 1992). The T3 binding capacity decreased twice during the differentiation of O-2A cells. These progenitors expressed α1, α2 but not β1 T3R-mRNAs. OL and type-2 astrocytes expressed β1, α1 and α2 T3R-mRNAs. The level of α2 T3R-mRNAs was twice higher in OL or type-2 astrocytes than in O-2A progenitors, whereas the level of the α1 T3R-mRNAs which correspond to the predominant mRNAs coding for functional T3Rs, remained unchanged. Our study indicates that α1, and α2 T3R-mRNAs are expressed in all cells of the OL lineage whereas β1 T3R-mRNAs are only expressed in OL and type-2 astrocytes. The temporal expression of the different subtypes of T3Rs suggest specific functions for α1 and β1 T3Rs during glial cell differentiation.

EVIDENCE FOR DOPAMINE D2 RECEPTOR mRNA EXPRESSION BY STRIATAL ASTROCYTES IN CULTURE; IN SITU HYBRIDIZATION AND POLYMERASE CHAIN REACTION STUDIES

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The expression of dopamine D2 receptor mRNA in 3 week cultured rat striatal and cerebellar astrocytes was examined by in situ hybridization (ISH) and polymerase chain reaction (PCR). In this culture, neurons were absent since neuronal specific enolase positive cells were not observed. Cells double-labelled for glial fibrillary acidic protein (GFAP) immunohistochemistry and dopamine D2 receptor mRNA (ISH) provide evidence that striatal but not cerebellar astrocytes express the dopamine D2 gene in vitro. These results were confirmed by PCR studies. As judged by GFAP immunostaining and morphology of the cells, this gene is almost exclusively expressed by astrocytes type 1. The expression of dopamine D2 mRNA by striatal astrocytes in vitro thus provides evidence for the presence of dopamine D2 receptors in these glial cells. This had previously been suggested from ligand binding studies, but the dopaminergic nature of the binding to striatal astrocytes was not clear.

Our results using a molecular biology approach suggests that striatal dopamine may modulate the functions of striatal astrocytes.

PURINE RELEASE FROM RAT GLIAL CULTURES IS MODULATED BY AGENTS AFFECTING CELL Ca2+ AVAILABILITY.

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It has been reported that cultured astrocytes release purines either at rest or under different kinds of stimuli. Purines, acting via their specific receptor sytes, are known to exert a variety of actions within the CNS including inhibition of transmitter release, increase of the O2 supply/demand ratio, regulation of blood flow. Either P1 or P2 purinergic receptors have been detected on astrocyte membranes and their activation has been coupled to Ca2+ ion movements. The present study was aimed to examine whether the modulation of astrocyte Ca2+ availability could influence purine outflow from dissociated primary glial cultures of rat striatum, in an attempt to better characterize its ionic requirements. Culture superfusion with a Ca2+ free medium + 0.5mM EGTA reduced the electrically evoked purine release. A stronger reduction was obtained by the Ca2+ channel blockers verapamil (30-120μM) and nitrendipine (0.01-0.1μM). The reduction caused by the culture co-superfusion with 0.1μM nitrendipine + 0.1μM CgTX was similar to that induced by nitrendipine alone. The Ca2+ channel activator BayK 8644 (0.1-10μM) was unable to affect the glial culture purine release. Neither the Ca2+ channel blockers nor the Ca2+ channel activator were able to modify the 45Ca2+ astrocyte uptake evaluated at rest and under the electrical stimulation. The effect of agents modulating the intracellular Ca2+ availability was also tested. TMB-8 and thapsigargin (Tg), drugs able to inhibit and increase the ion efflux form its internal stores respectively, and BAPTA/AM, able to chelate the cytoplasmatic free Ca2+, were used. TMB-8(10-30μM) and BAPTA/AM (50μM) decreased, whereas Tg (1-2μM) enhanced glial purine outflow (the increase being lower when the cells were pretreated with TMB-8). Measurements were also made by loading the glial cells with fura2/AM. Tg (0.5-2μM) was able to cause a rapid [Ca2+]i increase followed by a sustained plateau phase, which was lowered to basal levels by the addition of 5mM EGTA. Taken together these results suggest that neuronal N and L type VSCCs do not play a role in the modulation of the evoked purine release from dissociated primary glial cultures of rat striatum whereas purine outflow seems to be sustained by Ca2+ coming from intracytoplasmatic stores. When a stimulus is able to empty the ion stores, a refilling Ca2+ inward flux may be triggered, and the evoked purine release may thus become partially dependent on the extracellular Ca2+. Moreover, it seems worth underlining that, agents reported to block VSCCs seem able to modulate glial purine release with still poorly known mechanisms which deserve further investigations.

REGULATION OF NITRIC OXIDE-DEPENDENT cGMP FORMATION IN CEREBELLAR GLIAL CELLS

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There is evidence for the expression of a calcium-independent nitric oxide synthase (NOS) in astroglial cells upon induction with bacterial endotoxin (lipopolisaccharide, LPS) and cytokines. Additionally, we and others have presented data supporting the existence of a constitutive NOS in primary astroglial cultures. This activity is stimulated by noradrenaline (NA) in a calcium-dependent manner and the best response is observed in cerebellar astrocytes. In this work, we report that pretreatment of cerebellar astroglial cultures with the glucocorticoid dexamethasone (DXT; 1 μM, 18-20 h) which prevents NOS induction by LPS, increases almost three-fold the NO-dependent cGMP formation stimulated by NA (100 μM, 3 min). This effect is not due to an increased guanylate cyclase activity since sodium nitroprusside stimulation of cGMP formation is slightly decreased under the same conditions. On the other hand, NO-dependent cGMP formation induced by LPS (10 μg/ml, 18-20 h) is acutely inhibited 50-60 % by NA (100 μM, 3 min). A similar inhibition is observed with the calcium ionophore compound A23187 suggesting the involvement of calcium in this effect. These results indicate that constitutive and inducible NOS activities may be differentially regulated in astrocytes.

THE FATE OF OLIGODENDROGLIA IN CORONAVIRUS INDUCED DEMYELINATING ENCEPHALOMYELITIS

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The pathogenetic mechanisms involved in tissue damage in coronavirus induced demyelinating encephalitis are still controversal. They may include a direct virus induced cytolytic effect in infected cells, immune mediated destruction of virus infected cells or virus induced autoimmune reactions.
In our experimental model Lewis rats were intracerebrally inoculated with 800 PFU of the MHV-JHM virus between 3 and 8 weeks of age and developed demyelinating encephalomyelitis. In this study we have investigated the fate of oligodendrocytes in coronavirus induced primary demyelination. Oligodendrocytes were identified by immunohistochemical staining with monoclonal antibodies against MBP, PLP, CNP and MOG. In addition in situ hybridization was done for the detection of MBP, PLP, CNP and MOG mRNA. Degeneration of oligodendrocytes was identified by nuclear morphology and by visualization of DNA fragmentation by in situ nick translation.
Our results indicate that oligodendrocytes are destroyed by two different mechanisms: by necrosis of virus infected cells as well as by apoptosis of uninfected cells. This indicate that immune system may induce demyelination by either elimination of virus infected cells or in addition by bystander mechanisms.

ANALYSIS OF FOS AND JUN FAMILY MEMBERS IN THE GROWTH AND DIFFERENTIATION OF THE OLIGODENDROCYTE-TYPE-2 ASTROCYTE (O-2A) CELL LINEAGE.

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The oligodendrocyte-type-2 astrocyte (O-2A) cell lineage is comprised of a progenitor cell that is capable of differentiating into an oligodendrocyte in SATO medium devoid of growth factors or a type-2 astrocyte in DMEM-10% FCS. In the presence of PDGF and bFGF O-2A progenitor cell self renewal is promoted but differentiation is inhibited[1]. To study the molecular mechanisms involved in glial differentiation it is necessary to create cell lines so that bulk cultures can be created of the progenitor cell and its differentiated progeny. For this reason O-2A progenitor cell lines have been created by retroviral infection of PDGF and bFGF stimulated O-2A cells with the temperature sensitive mutant (ts) gene of the large T antigen[2] and the c-myc gene. The ts T cell line (at the non permissive temperature) and the c-myc cell line have enhanced viability and are able to differentiate into oligodendrocytes and type-2 astrocytes in a similar manner to primary cultures of O-2A progenitor cells.
C-fos and c-jun proteins have been implicated in the regulation of oligodendrocytic differentiation[3]. These proteins are members of inducible gene families whose protein products can form an array of homodimeric and heterodimeric complexes that function as transcriptional regulators by interacting with DNA sequences related to the AP-1 and CRE motifs. To study the role of these proteins in the differentiation of the O-2A cell lines immunoprecipitation studies have been carried out with several fos and jun antibodies. In the tsT cell line at the non permissive temperature it was seen that there was high expression of c-fos and bound jun in the progenitor cells which decreased in oligodendrocytes and type-2 astrocytes. In contrast in the c-myc cell lines c-jun expression was absent in progenitor cells and present in oligodendrocytes and type 2 astrocytes while Jun B expression is present in all three cell types. Further experiments are in progress to clarify the expression of the individual fos and jun family members in these two cell lines.

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INTERLEUKIN-1β IN LEWIS RAT BRAIN DURING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IS PRESENT IN MICROGLIAL CELLS BUT NOT IN ASTROCYTES.

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Interleukin-1β (IL-1) is a cytokine which exerts many biological effects during inflammation. In the present study experimental autoimmune encephalomyelitis (EAE) was induced in Lewis rats by inoculation of guinea pig spinal cord homogenate. During the various stages of EAE, the presence of IL-1 in the brain was investigated using immunocytochemistry at both the light and ultrastructural level with a rabbit polyclonal antiserum against rat IL-1β. Ten days after immunization, IL-1-immunoreactivity was found in brains of animals which at this time showed mild clinical signs. Outside the blood-brain barrier, IL-1 was localized in meningeal macrophages and perivascular cells. Within the brain parenchyma, IL-1-immunoreactivity was found in perivascular lesions in infiltrated macrophages and in activated microglia. On day 13 (full blown EAE) the number of lesions with IL-1-positive cells had increased. In the remission phase (day 25), lesions with IL-1-positive cells could still be detected but were less pronounced as compared to day 13. Other presumptive IL-1 producing cell types like endothelial cells or astrocytes were, at none of the various stages, found to stain for IL-1.

VOLTAGE-GATED CURRENTS OF GLIAL CELLS IN THE JUVENILE RAT HILUS

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The electrophysiological properties of neurons in the hippocampal formation have been described in great detail, while their glial partners were not under study until recently. We investigated the voltage-gated currents of glial cells in the hilar region of the dentate gyrus from juvenile rats (P6-P14) using the nystatin whole-cell mode of the patch-clamp technique in acute slices. With a potassium containing pipette solution, we found two different types of K^+ currents. In 19 out of 34 cells there was a passive time-independent conductance, which showed inward as well as outward rectification in different cells. In 15 out of 34 cells a delayed rectifier K^+ current was seen, partly in combination with A type- and inward rectifier - K^+ currents, and Na^+ currents. The glial cells in the hilus express current types previously described in glial cells of the CA1 stratum radiatum (Steinhäuser *et al.*; 1992; Eur. J. Neurosci. 4:472-484). A subpopulation of the cells with passive currents were identified later on (Steinhäuser *et al.*; 1994; Hippocampus, in press) as GFAP-positive astrocytes, while cells with a "complex" delayed rectifier pattern are presumable glial precursor cells. In contrast to the study in CA1 stratum radiatum, we could not find cells with electrophysiological characteristics of oligodendrocytes (see Berger *et al.*; 1991; J. Neurosci. 11:3008-3024) in the hilus, probably due to the late myelination of this area (Berger and Frotscher; 1994; J. Neurocytol., in press).

SINGLE CHANNEL PROPERTIES IN GLIAL PRECURSOR CELLS OF THE MOUSE CORPUS CALLOSUM SLICE

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In the corpus callosum of mice, myelination starts about eight to ten days after birth. At that time in development, glial precursor cells mature into oligodendrocytes. Concommitant with this differentiation step, the membrane channel pattern changes dramatically: while glial precursor cells are characterized by delayed rectifying outward currents, oligodendrocytes express passive non-voltage dependent currents (1). To assess the underlying single channel properties of the membrane currents, we used the patch-clamp technique in the cell attached mode to record single channel currents from cells of P4-P7, thus of glial precursor cells. The majority of the channels (49 out of 61) were outwardly rectifying and activated at a membrane potential which was about 42 mV (0 to 78 mV) positive to the extrapolated reversal potential. The extrapolated reversal potential was 6 mV positive to resting potential and points to a K^+ selectivity. The single channel conductance, however, varied in a large range, namely between 4 and 66 pS (mean 19 pS) suggesting the presence of a family of channels with similar kinetics but different conductive properties. In addition, we could also detect inwardly rectifying K^+ currents (N=5). The properties of the cells from which single channel data were sampled were further characterized by subsequently recording whole cell currents and staining cells with Lucifer yellow via the patch-pipette. This procedure verified that we indeed recorded from glial precursor cells. The characterization of K^+ channels is of interest beyond its biophysical aspect since K^+ channels can play an important role in the control of proliferation (2).

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TRANSMEMBRANE AND INTRACELLULAR TRANSPORT OF THYROID HORMONES IN CULTURED ASTROCYTES

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Astrocytes are target cells of thyroid hormones (THs), which contain L-triiodothyronine (L-T_3) nuclear receptors and a 5'-deiodinase that activates L-thyroxine (L-T_4) to L-T_3. We have studied, in cultured rat brain astrocytes, the pathways which can regulate the access of THs to their intracellular targets.

We found that the membrane transport of THs is carrier-mediated and obeys simple Michaelis-Menten kinetics. The transport constants (Kt) are: 0.5 µM for L-T_3 and 1 µM for L-T_4. The specificity of the transport system for TH analogues is L-T_3 > L-T_4 > reverse L-T_3 (rT_3) > diiodothyronine (L-T_2) > D-T_3 > triiodothyroacetic acid (triac).

We report that the transport of THs is related to the transport of aminoacids. First, a Na^+-independent aminoacid transport system with high-affinity for leucine and tryptophan (Kt ≈ 10 µM), similar to System L_1, is characterized. Second, this system is competitively inhibited by L-T_3 (Ki ≈ 2-3 µM). TH analogues inhibit similarly the System L_1 and the TH transport system, indicating that both systems are stereospecific.

The intracellular traffic of THs involves cytosolic binding proteins. We report the presence of a high-affinity (Kd ≈ 0.3 nM) L-T_3-binding protein activated by NADPH. The specificity of the protein is : D-T_3 > L-T_3 > L-T_4 > triac > rT_3 = L-T_2. The binding entity was identified by photoaffinity labelling with [^{125}I]L-T_3 as a 35-kDa polypeptide.

In conclusion, we have characterized in cultured astrocytes various systems participating in intracellular TH traffic. Astrocytes are suitable to study the interaction between proteins involved in the TH signalling pathways.

GABA UPTAKE INTO ISOLATED MÜLLER GLIAL CELLS OF THE GUINEA PIG RETINA

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The GABA uptake system was investigated in acutely isolated Müller cells (MC) of young adult guinea pigs by means of the whole-cell voltage-clamp technique. To this purpose the MC K^+ conductance was strongly suppressed by extracellular Ba^{2+} (1 mM). Thus, the input resistance of MC increased to 1.1 GΩ. Under these conditions, GABA evoked inward currents at membrane potentials negative to 0 mV, were carried by a GABA transporter (GAT). Since MC were insensitive to application of muscimol and baclofen, involvement of $GABA_A$ and $GABA_B$ receptors can be excluded. Likewise, the GAT hypothesis is strongly supported by suppression of the currents when the normal Na^+ gradient is destroyed by replacement of extracellular Na^+ by choline. Application of the $GABA_C$ agonist CACA (0.1 mM) evoked an inward current, that was also suppressed when external Na^+ was removed. Thus, this compound is a substrate for the MC GAT. Nipecotic acid (0.1 mM) and β-alanine (0.1 mM), known to be competitive blockers of GATs, are also transported by the MC GAT. They produce 65% and 42%, respectively, of the currents elicited by the same GABA concentration. The GABA dose response curve revealed a Michaelis-Menten kinetic with a K_m of 5 µM and a saturating concentration of 100 µM, both at a membrane potential of -80 mV. Removal of intracellular K^+ is not suppressing the GAT function, as is reported for glutamate uptake into MC.

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ASTROCYTIC M-CSF AS COFACTOR REQUIRED BY MICROGLIA FOR IFNγ-INDEPENDENT ANTIGEN PRESENTATION

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During development with exogenous GM-CSF microglia from murine brain cell cultures aquire highly efficient antigen presenting function. Antigenic activation of helper T lymphocytes by this type of microglia occurs without any preactivation by IFNγ. However, isolation of microglial cells from concomitant astrocytes in mixed glial culture initiates a loss of stimulatory capacity despite prolonged exposure to GM-CSF. To investigate a presumable influence of astrocytes during microglia differentiation both populations were separately cultured in double-chambers divided by a 0.4µm porous membrane. In this system a 5-day-exposure of microglia to GM-CSF and soluble products of feeder astrocytes preserved antigen presenting capacity similar to that of the corresponding not separated cells. Furthermore, the medium conditioned by astrocytes was able to replace the effects of both exogenous GM-CSF and cocultured astroglia.
Astrocyte-derived M-CSF was identified to be the cofactor required by GM-CSF-grown microglia since (i) the soluble factor which preserves microglial activation was copurified with astrocytic M-CSF, (ii) its effect was abolished by preincubation with a neutralizing monoclonal anti murine M-CSF antibody and (iii) recombinant murine M-CSF totally substituted the activity contained in astrocytic supernatants.
By their capability to produce both M-CSF and GM-CSF astrocytes are suggested to regulate microglial antigen presentation function through a pathway uncoupled from IFNγ.

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NON-NEURONAL EXPRESSION OF THE BDNF RECEPTOR TRUNCATED *trk*B

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Brain-derived neurotrophic factor (BDNF) is known primarily for its role as a protein allowing the survival of neurons. This biological effect involves the activation of the tyrosine kinase receptor *trk*B. In mammals, a truncated form of the *trk*B receptor lacking the tyrosine kinase domain has been described in ependymal and glial cells, but the binding properties of truncated *trk*B have not been described in detail. In the course of studies aimed at localising the sites of *trk*B expression in the developing chick embryo, we found high levels of *trk*B mRNA in all leptomeningeal cells surrounding the developing spinal cord and brain, as well in some mesenchymal derivatives. In some non-neuronal cells, *trk*B receptor expression was found adjacent to sites where BDNF-mRNA expressing cells could be localised. Northern blot and PCR analyses indicated that the *trk*B mRNA expressed in leptomeningeal cells codes for a receptor lacking the tyrosine kinase domain, and Western blot analysis with an antibody directed against an extracellular sequence of *trk*B revealed a protein of 88 kd, consistent with the size of a truncated *trk*B receptor. As a previous study with full-length chick *trk*B expressed in a non-neuronal context (A293 cells) had indicated a dissociation constant of 10^{-9} M for BDNF (as opposed to 10^{-11} M found on BDNF-responsive peripheral neurons), we were interested to determine the binding characteristics of truncated *trk*B in the context of leptomeningeal cells. The results of preliminary experiments both on sections and on freshly dissociated cells indicate the presence of high affinity BDNF binding sites. These and other findings suggest that non-neuronal cells can bind BDNF through high affinity receptors, that truncated *trk*B is probably involved in the formation of these high affinity receptors, and that the formation of high affinity BDNF binding sites do not necessarily require a neuronal context. The biological function of such high affinity neurotrophin binding sites in leptomeningeal and other non-neuronal cells is unclear, but could help creating sharp boundaries regulating neurotrophin availability during development.

A NEW OLIGODENDROCYTE SPECIFIC PLASMA MEMBRANE SURFACE PROTEIN IDENTIFIED BY A MONOCLONAL ANTIBODY PRODUCED IN VITRO

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In the central nervous system (CNS), it is likely that myelination calls for complicated interactions between oligodendrocytes (OL) and axons. In order to identify new glycoproteins involved in myelin membrane formation, we have generated monoclonal antibodies (MAbs) starting with a wheat germ agglutinin glycoprotein fraction isolated from bovine CNS myelin. In this study, we describe a novel MAb (C1G5F2) produced by *in vitro* immunization of mice splenocytes. Immunohistochemical reactions of C1G5F2 were investigated on fixed rat brain sections during the active period of myelination. It was observed that C1G5F2 antigen deposition in myelin sheaths followed a caudo-rostral gradient. The first immunolabeled myelin fibres were detected within the pons at day 14 postnatally, and the white matter areas in the cerebrum were clearly immunopositive after the third week. No positive cell bodies of OL were observed throughout the brain structures. The other neural cell types were also immunonegative. C1G5F2 was not present in the peripheral nervous system and in other peripheral tissues. Immunohistochemical detection of C1G5F2 epitope was also investigated on unfixed brain sections of several animal species; its expression appears restricted to the aves and mammalian classes. The C1G5F2 MAb bound mainly to the extracytosolic membrane surface of the processes of live mature cultured oligodendrocytes derived from newborn rat brain. The MAb was unreactive with live or fixed astrocytes and neurons maintained in culture. Immunopurification of the epitope containing antigen was achieved following two different immunochemical procedures. A main protein band of 54-58 kDa was detected after SDS-PAGE. We postulate that this antigen, which is expressed on the extracytosolic surface of cultured OL and found on myelin sheaths in the brain, may possibly serve as an antigenic target in certain idiopathic diseases that are restricted to CNS myelin.

Consumption of Branched-chain Amino Acids and Glycine by Astroglia-rich Rat Brain Cell Cultures

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Leucine, isoleucine and valine, the three branched-chain amino acids (BCAA), are known to be rapidly metabolised in the brain. In order to allocate to a certain cell type the capacity to use BCAA for energy production, as a first system to be investigated astroglia-rich primary cultures derived from rat brain were used in metabolic studies.
HPLC analysis revealed that the cells remove BCAA from the culture medium at high rates, even though sufficient substrates for the generation of energy, such as glucose were present. Further more, it was observed that glycine is completely cleared from the medium within a few days.
Auestad et al. [1] have shown that astrocytes produce ketone bodies through ß-oxidation of fatty acids and that they release these ketone bodies into the culture medium. Taking into consideration that the only way leucine can be degraded is to acetyl-CoA and acetoacetate, it seems to be obvious that the catabolism at least of leucine should lead to ketone bodies which would most likely be exported. The ketone bodies would then serve as energy substrates for neighbouring cells like neurons and oligodendrocytes [2]. Indeed, generation of ketone bodies is observed in these cultures, albeit their source remains to be elucidated.

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AREAS OF DEMYELINATION REPAIRED BY MOUSE OLIGODENDROCYTES AND RAT TYPE-1 ASTROCYTES CREATE A NEW MODEL OF IMMUNE-MEDIATED DEMYELINATION

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We have created a new model of immune-mediated demyelination in which an infammatory response is directed against mouse oligodendrocytes which have been established within the spinal cord of immunosuppressed rats. Areas of demyelination created by intraspinal injection of ethidium bromide are transplanted with cell suspensions composed of isogeneic type-1 astrocytes and mouse oligodendrocyte lineage cells. Recipient animals are imunosuppressed for 14 days during which time mouse oligodendrocytes remyelinate rat axons and, together with rat astrocytes, reconstruct the glial enviroment of the lesion. Following cessation of immunosuppression, the mouse oligodendrocytes are rejected and this second wave of demyelination is rapidly followed by host-mediated oligodendrocyte remyelination. These findings indicate that the events associated with a self-limiting episode of inflammation do not inhibit remyelination.

DYNORPHIN 1-17 DELAYS THE VASOPRESSIN INDUCED MOBILIZATION OF INTRACELLULAR CALCIUM IN CULTURED ASTROCYTES FROM THE RAT NEURAL LOBE

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Opioid peptides are present in nerve terminals in the rat neural lobe where they partially coexist with vasopressin. Morphological findings suggest that these neuropeptides are released onto pituicytes (1-3), which is in agreement with a possible role for the pituicyte in oxytocin and vasopressin release from the neural lobe.
Pituicytes in culture respond to vasopressin with a mobilization of calcium from intracellular stores. In the present study this vasopressin induced increase in intracellular free calcium levels was both delayed and decreased by pre-exposure to dynorphin 1-17, while dynorphin 1-17 by itself did not affect basal calcium levels (4,5). All effects of dynorphin 1-17 could be blocked with naloxone.
The present results suggest that opioid receptors are present on pituicytes and are coupled to a second messenger pathway by which opioid peptides may inhibit inositol phosphate dependent calcium mobilization by other neuropeptides, such as vasopressin.

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AMPA/kainate receptor activation in murine oligodendrocyte precursor cells leads to activation of a cation conductance, Ca^{++} influx and K^+ channel blockade

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During the last ten years it has been established that glial cells express a large repertoire of neurotransmitter receptors. In this study we have characterized the properties of a AMPA/kainate receptor in glial precursor cells from cultures of mouse brain using the patch-clamp technique to measure ligand-activated currents and the fura-2 imaging system to determine changes in cytosolic Ca^{++}. Precursor cells were identified by their characteristic morphology, positive staining with the O4 antibody and a defined set of voltage-gated currents as described by Sontheimer et al. (1989). The ligands L-glutamate, AMPA, kainate and domoate elicited inward currents at a holding potential of -70 mV. The expression of an AMPA/kainate receptor by glial precursor cells was further substantiated by the reversible blockade of the kainate and AMPA responses by CNQX. The different glutamate receptor agonists led to the activation of a cationic conductance as revealed by analysing the reversal potential. Receptor activation was accompanied by two additional responses: (1) an increase in cytosolic Ca^{++} mediated by depolarization and a subsequent activation of voltage-gated Ca^{++} channels and (2) a transient blockade of the (resting) delayed rectifying K^+ current, but not the A-type K^+ current. The blockade of the K^+ current was not due to the increase in Ca^{++}, since it was also observed in Ca^{++}-free bathing solution. In contrast, the blockade was mediated by the influx of Na^+ via the receptor channels, since the K^+ channel block after kainate receptor activation was not observed in a Na^+-free bath or when cytoplasmic $[Na^+]$ was elevated.
Mature oligodendrocytes, in contrast, responded not at all or only weakly to glutamate or related ligands. We conclude that glutamate activates a complex pattern of physiological events in the glial precursor cells and it is likely that such mechanisms play a role during the differentiation process of these cells.

Sontheimer et al. (1989) Neuron 2, 1135-1145.

Adult rat oligodendrocyte progenitor cells express a repertoire of voltage and ligand gated ion channels distinct from their perinatal counterpart

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Perinatal oligodendrocyte progenitor cells derived from embryonic or postnatal central nervous system (CNS) and grown in vitro express a pattern of ion channels that is distinct from mature oligodendrocytes and other cell types of the CNS (1,2). In the present study, we used the whole cell patch clamp technique and the fura-2 based calcium imaging system to study ion channel expression of cultured adult oligodendrocyte progenitor cells derived from 19 month old rat optic nerve; these cells differ in several respects from perinatal progenitor cells in vitro (3).
Adult progenitor cells were grown in PDGF-containing serum-free medium and were identified by their characteristic unipolar morphology and in some cases also by a positive staining with the A2B5 mAb or O4 mAb. The adult progenitor cell membrane is dominated by both delayed and inwardly rectifying K^+ currents. In comparison to the perinatal cells, the inwardly rectifying currents were often as large as the outward delayed rectifying K^+ currents. In addition, most cells showed no or only small A-type K^+ currents. In contrast to perinatal oligodendrocyte precursor cells from optic nerve (1) but similar to murine cortical oligodendrocyte precursor cells (4), adult progenitor cells expressed Ca^{++} channels, as determined electrophysiologically and with the calcium imaging system. Like perinatal cells, the adult cells expressed Na^+ currents, $GABA_A$ receptors and a kainate-type glutamate receptor. The results suggest that adult progenitor cells share many common electrophysiological features with both, immature oligodendrocytes and precursor cells (2,4).

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LIPOPOLYSACCHARIDES INDUCE AN UP-REGULATION OF THE IL-1 mRNA AND A DOWN-REGULATION OF THE GFAP-mRNA IN CULTURED MOUSE ASTROGLIAL CELLS.

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Cytokines regulate the immune response in the CNS. The molecular mechanisms through which the expression of their genes is controlled are as yet not fully elucidated.. Since these mediators can be induced by treatment of cells by bacterial lipopolysaccharides (LPS) we investigated how astrocytes react to LPS using enriched astroglial primary cultures at both, their proliferative , and morphologically differentiated states .
During postnatal astroglial proliferation, LPS induces a dose and time dependent inhibition of 3H-Thymidine incorporation. This effect is correlated with a small decrease in the GFAP level. During later astroglial maturation, LPS induces a dose and time-dependent inhibition of 3H-Thymidine incorporation on the remaining proliferating astrocytes. This effect is correlated with a dose and time-dependent down-regulation of the GFAP-mRNA and a transitory induction of the IL-1-mRNA, whereas expression of the IL-6 and IL-8 messages were undetectable. The GFAP level did not decrease significantly.
We have shown that a down-regulation of the GFAP encoding message and a stabilization of GFAP are associated with astroglial differentiation and preservation of cell shape. Further investigations are necessary to clarify how LPS affects the differentiation and maturation of astrocytes and what type of relation may exist between the IL-1 message induction and the astroglial cytoskeleton network.

REGULATION OF INTRACELLULAR pH IN CULTURED RAT OLIGODENDROCYTES: EVIDENCE FOR Na^+/H^+ AND HCO_3-DEPENDENT ACID EXTRUSION MECHANISMS

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Intracellular pH (pH_i) was measured at 37 °C in mature rat cerebellar oligodendrocytes dissociated in culture using microfluorescence analysis of intracellularly trapped BCECF (2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein). Cells were identified by anti-galactocerebroside and OL1 antibodies. The steady state pH_i was 7.04 ± 0.06 (mean ± SEM, n = 30) when cells were perfused by a bicarbonate buffered solution (25 mM bicarbonate, 5% CO_2, pH 7.40, 37 °C). Replacing all the external chloride with gluconate does not modify this steady state value. When cells were perfused by HEPES-buffered solution (pH 7.40), the steady state pH_i was 7.06 ± 0.05 (n = 30), a value not significantly different than in bicarbonate solution. After an intracellular acid load (NH_4 prepulse technique) pH_i recovered to its initial value only in the presence of external sodium. This recovery was completely inhibited by amiloride (1.5 mM) in HEPES-buffered solution. In contrast pH_i recovery was unaffected by amiloride or stilbene derivatives (DIDS or SITS, 0.5 mM) when cells were perfused by the bicarbonate-buffered solution. These results indicate that Na^+/H^+ and bicarbonate-dependent acid extrusion mechanisms are involved in pH_i regulation of mature cerebellar oligodendrocytes, the nature of the later mechanism is under study. No evidence was found for the existence of a Cl^-/HCO_3 exchanger in these cells.

FIBROBLAST GROWTH FACTORS AND INSULIN-LIKE GROWTH FACTORS SUPPORT SCHWANN CELL PRECURSORS IN VITRO

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We have isolated Schwann cell precursors from rat limb nerves at embryonic day 14–15. Developmentally, these cells lie between the neural crest and Schwann cells. The transition from precursor cells to Schwann cells occurs abruptly between embryonic days 15 and 17. Although Schwann cell precursors share certain characteristics with neural crest cells, they are morphologically and phenotypically distinct, and able to generate Schwann cells in culture under appropriate conditions.

These Schwann cell precursors die by apoptosis in culture unless growth factors or neurites are present to rescue them. Here we show that at least three members of the fibroblast growth factor family, in combination with insulin-like growth factors, can prevent the death of these cells in culture. This rescue does not need exogenous cAMP elevating agents and does not lead to DNA synthesis.

DIFFERENTIATION OF RAT TELENCEPHALON GLIA CELLS UNDER HYPOXIA CONDITIONS

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A study was conducted on the influence of the mother's hypoxia on the early development of telencephalon glia cells of rat embryos. In the course of the first 12 days (E1-12) of pregnancy haemorrhages in the uterus, blastocyst degenerations, pathologic chorion vesicle development, defects of neurulation etc. were observed. In the present study our attention was directed to the "healthy looking" embryos. On the E13 of prenatal life the developing telencephalon vesicle wall was formed by the ventricular zone. The neuroepithelial cells were arranged as a pseudostratified epithelium and beneath its internal surface, mitotic cells were present. Some cell elements with mitotic figures could be seen to have intensive anti-vimentin staining in the cytoplasm while the other mitotic cells did not display any positivity. Radially arranged processes of daughter cells span the whole layer. They were labelled with anti-vimentin antibody similarly to the widened parts of the processes which touched the capillary wall or constituted the lamina limitans interna and externa. Telencephalon vascularization originated from the perineural plexus. On the E15 the marginal zone was developed. Triangle shaped end feet of still undifferentiated glioblasts constituted primitive lamina limitans "gliae" superficialis. The whole layer of expanding tissue was already vascularised. At E18 cortical plate was differentiating. Retardation of the tissue development was evident. GFAP positive intermediate filaments were found in the processes of glial cell precursors. Single cells of microglia character were present in the tissue.

EXPRESSION OF A SODIUM INDEPENDENT NEUTRAL AMINO ACID TRANSPORTER FROM ASTROGLIA CELLS IN *XENOPUS LAEVIS* OOCYTES.

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Astroglia cells have a major function in nutrition and energy metabolism of the brain. Cultured astroglia cells consume large amounts of neutral amino acids. One of the transporters which is responsible for this uptake is the system L amino acid transporter. As a first step in cloning of system L we characterized the responsible poly $(A)^+$ RNA by expression in *Xenopus laevis* oocytes. Poly $(A)^+$ RNA isolated from rat brain astroglia and C6-BU-1 cells induced sodium dependent and sodium independent isoleucine transport in injected oocytes. The sodium independent transport process was inhibited by 2-aminobicyclo-[2,2,1]heptane-2-carboxylic acid (BCH) but not by methyl-aminoisobutyric acid (MeAIB) or lysine. A K_m of 119 µM was determined for the sodium independent transport of isoleucine. This is in accordance with expression of a system L like transporter. Sodium dependent isoleucine could also be detected in *Xenopus laevis* oocytes. We could assign this activity tentatively as system ASC dependent. We found a fraction of size selected poly $(A)^+$ RNA which induced sodium independent isoleucine transport activity in oocytes. This fraction will be used for construction of a cDNA library.

REGIONAL PECULIARITIES OF GLIA-MATRIX-NEURON INTERFACE IN THE RAT BRAIN

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The neuronal microenvironment may substantially be determined by glial cell processes and the extracellular matrix. Its high content in polyanionic proteoglycans seems to be especially characteristic for two forms of the neuron-glia interface: nodes of Ranvier and the perineuronal nets.
In this study, proteoglycan components were electron microscopically detectable in the rat neocortex, cerebellar nuclei, medial septum and thalamic reticular nucleus using the N-acetylgalactosamine-binding plant lectin *Wisteria floribunda* agglutinin (WFA). Lectin-binding material was observed in dilated extracellular spaces around neuronal somata and dendrites (perineuronal nets), axon initial segments and unmyelinated axonal bundles. The labelled areas in perineuronal nets varied in shape and size, being very large (up to 1µm) in cerebellar nuclei and some of the cortical non-pyramidal neurons. They were distinct but smaller in the septal region and rather slender in the thalamic reticular nucleus. Astrocytic profiles, often connected by gap junctions, were found to be adjacent to the perisynaptic and periaxonal extracellular matrix. The glia-matrix-neuron interface was clearly developed in neurons with high density of axo-somatic contacts.
These findings suggest that an enlarged extracellular matrix volume may be supportive for astrocyte functions. Similar to the perinodal space at nodes of Ranvier, this specialized microenvironment may provide a high ion buffering capacity in close vicinity to distinct types of neurons which might be required for high frequent or tonic discharge pattern.

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INTRACELLULAR pH REGULATION IN CULTURED RAT CEREBELLAR ASTROCYTES

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The prominent role of glial cells for the regulation of brain microenvironment is well established. We have investigated the regulation of intracellular pH (pH_i) and bicarbonate-dependent membrane currents in cultured astrocytes from neonatal rat cerebellum, using the pH-sensitive fluorescent dye 2',7'-bis(carboxyethyl)-5,6-carboxyfluorescein-acetoxymethylester (BCECF-AM) and the whole-cell patch-clamp technique. Introducing 5 % CO_2/ 24 mM HCO_3^- (pH 7.4) to a HEPES- buffered, nominally CO_2/ HCO_3^--free saline (pH 7.4) resulted in a brief intracellular acidification, from which the cells rapidly recovered, and an outward current. Removal of CO_2/HCO_3^- produced a transient intracellular alkalinization followed by a prominent acidification with a slow recovery, and a transient inward current. Reducing the external pH from 7.4 to 6.6 produced a faster and larger decrease of pH_i and inward current in the presence of CO_2/HCO_3^- than in its absence. Amiloride (2mM) caused a significant decrease of pH_i in nominal CO_2/HCO_3^--free saline, but introduction of CO_2/HCO_3^-- in the presence of amiloride produced a reversible intracellular alkalinization. The CO_2/HCO_3^--dependent alkalinization and outward current were reversed after the removal of external Na^+. The stilbene 4,4' - diisothiocyanatostilbene -2,2'-disulfonic acid (DIDS, 0.3 mM) reduced the pH_i recovery from the CO_2/HCO_3^--evoked transient acidification, and blocked the prominent intracellular acidification upon removal of CO_2/HCO_3^-; both effects were also evident in the absence of external Cl^-. The results suggest that the CO_2/ HCO_3^--dependent current is due to a reversible, electrogenic Na^+-HCO_3^- cotransporter, which helps to regulate pH_i in these cells. In addition, a prominent Na^+/H^+ exchanger, and presumably a moderate Na^+-dependent Cl^-/HCO_3^- exchanger, contribute to extrude acid equivalents from these astrocytes.

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Antibody activities in glioma cyst fluids

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Immunoglobulins and blood-group antigens have been observed in cyst fluids which are associated with 25 to 54% of astrocytomas. Anti-normal brain antibodies were studied in glioma cyst fluids. Cyst fluids were obtained by stereotaxic puncture from 34 patients with cystic gliomas. Immunoglobulins were analyzed by immuno-histochemistry, immunowesternblotting, concentration measures, and isoelectrofocalization. 80% of cyst fluids stained astrocytes and/or microvessels in non-tumoral white matter. In white matter extracts, cyst fluids recognized five immunoreactive bands having apparent molecular weights of 50 to 75 kDa. Although cyst proteins could be of systemic origin, isoelectrofocalization analysis suggests an additional local immune response. Antibody activities against perivascular astrocyte fibers and microvessels may play a role in the control of BBB permeability and they may be related to remote tumor effects, such as peritumoral edema.
Anti-tumoral activities are also studied in these cystic fluids. First results seem to show a tumoral tissue specificity of antigenicity, and anti-tumoral activity specificity of cyst fluids depending on tumoral grade.

GENETIC SUSCEPTIBILITY OF THE MOUSE TO THE PERSISTENT INFECTION BY THEILER'S VIRUS

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The DA strain of Theiler's virus, a picornavirus, causes chronic inflammation and demyelination in the spinal cord of susceptible mice. This natural viral disease is a model for Multiple Sclerosis. Inbred strains of mice show various degree of susceptibility to this disease. To simplify the genetic analysis of this complex disease, we have been studying the genes which control viral persistence, a prerequisite for demyelination. The amount of viral RNA in the spinal cord was measured with a semi-quantitative dot blot assay. Major differences in the levels of viral RNA were observed among 17 mouse strains tested. These differences could be explained by the interaction of at least two groups of loci. One locus is localized in the *H-2D* region. The *b* haplotype confers resistance in a dominant way. We have demonstrated that the gene responsible for resistance is the class I gene H-2D^b itself. Susceptible FVB mice were made transgenic for the H-2D^b gene and became resistant. A second group of loci is localized outside the MHC. Its susceptible allele is present only in the SJL/J strain and is dominant. A genetic analysis on 79 F1 (B10.S x SJL/J) x B10.S backcross mice using 89 polymorphic microsatellites showed that a second locus controlling viral persistence is localized close to *Ifg* on Chr10 and a third one may be localized close to *Mbp* on Chr18. Resistant mice became highly susceptible after inactivation of the gene coding for the receptor to inferferon gamma. The IFG gene is therefore a candidate gene for the control of viral persistence.

ASTROCYTE RESPONSE TO GABA IN THE DEVELOPING RAT OPTIC NERVE

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Recently, it was shown that GABA caused a depolarization in the neonatal rat optic nerve which attenuated during development (Sakatani et al., 1992, Proc. Roy. Soc. B247, 155). The aim of this study was to determine whether astrocytes are the source of this response to GABA. Optic nerves from rats aged 1-25 days old were isolated and maintained in a brain slice chamber. Electrophysiological recordings were made with intracellular microelectrodes and all cells were injected with HRP for subsequent morphological identification as astrocytes. Identified astrocytes responded to 1 mM GABA with a depolarization which diminished with age from a mean (± SEM) of 13 ± 2 mV at P1, 8 ± 1 mV at P10 and 0 mV at P21. The depolarization was characterised by an initial peak which was not sustained but fell to a plateau potential. The GABA response was mimicked by the $GABA_A$ receptor agonist muscimol but not by the $GABA_B$ receptor agonist baclofen. Thus, immature rat optic nerve astrocytes respond to GABA in situ by a depolarization which is mediated via $GABA_A$ receptors. This effect is attenuated with age and may be important in mediating axon-glial interactions during development.

ASTROCYTE ASSOCIATION WITH NODES OF RANVIER IN THE MOUSE OPTIC NERVE

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Astrocytes are implicated in the function of nodes of Ranvier because their perinodal processes form contacts with the axonal membrane at nodes. It is maintained that in the optic nerve these processes emanated from a specialised perinodal cell, the type-2 astrocyte (ffrench-Constant & Raff, 1986, Dev. Brain Res. 3, 371; Miller et al., 1989, Eur. J. Neurosci. 1, 172). The aim of this study was to determine the precise relationship between astrocytes and nodes in the mouse optic nerve, by correlative confocal and electron microscopic analysis of astrocytes dye-filled with lysinated rhodamine dextran or horseradish peroxidase. All astrocytes analyzed had processes which contacted nodes along their length and terminated as end-feet on blood vessels or at the subpial glia limitans. Astrocytes also formed multiple nodal contacts via a system of collateral branches which passed longitudinally along the axonal axis, subsequently ending at nodes. It is concluded that, in the mouse optic nerve, there is a single population of astrocytes which perform multiple functions, and perinodal processes are not formed by a specialised cell type.

INDUCTION OF INTERLEUKIN-1ß mRNA AFTER FOCAL CEREBRAL ISCHAEMIA IN THE RAT

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The expression of interleukin-1ß (IL-1ß) mRNA in the brain in response to cerebral ischaemia in rats was examined using *in situ* hybridization. Focal cerebral ischaemia was induced in spontaneously hypertensive rats by permanent occlusion of the left middle cerebral artery (MCAO). Whereas no IL-1ß mRNA could be detected in non-operated and sham-operated rats, middle cerebral artery occlusion induced the expression of IL-1ß mRNA within 15 min in the ischaemic brain regions prone to become necrotic after 1-2 days. The message appeared as spot-like signals, reached a peak after 3 hours and then declined to undetectable levels within 4 days. Additionally, a pronounced but brief induction of IL-1ß mRNA could be detected 1 h after MCAO in the meninges near the watershed zone. The results demonstrate that the inflammatory cytokine IL-1ß is induced in a time dependent way after brain ischaemia. The round-shaped morphology of the IL-1ß mRNA positive cells 48 h after MCAO indicates that these cells are invading macrophages or activated microglia.

ACTIVATED MICROGLIA EXPRESSES NITRIC OXIDE SYNTHASE (NOS) ACTIVITY IN QUINOLINATE LESIONED RAT STRIATUM: AN ULTRASTRUCTURAL STUDY

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Recently it has been reported that NO mediates neurotoxicity associated with excessive stimulation of the glutamate NMDA receptor subtype. NO is synthesized from L-arginine by the enzyme NO-synthase (NOS). Its constitutive isoform has been found to be present in a discrete population of neurons in the brain, while induceable form has been reported in macrophages. Both forms of the enzyme express NADPH-diaphorase (NADPH-d) histochemical activity which is also used as a marker for the NOS. While B_4-isolectin is an established marker for microglial cells, in the present study we examined expression of NADPH-d in isolectin B_4 positive microglia/macrophage in quinolinic acid (QUIN) lesioned rat striatum. In tissue affected by QUIN-induced cell damage a significant majority of the observed macrophages appeared to be NADPH-d positive, whereas the majority of NADPH-d labeled macrophages also showed IB_4 reactivity. Thus more than a half of the studied striatal macrophages were found to be double labeled. B_4-isolectin labeled cytoplasmic membranes with DAB precipitate, while well distinguishable formazan of the NADPH-d staining labeled the nuclear envelope, endoplasmic reticulum, Golgi apparatus and mitochondria indicating the ultrastructural location of the NOS. Based on present morphological observations it is concluded that QUIN-caused neurodegeneration stimulates expression of nitric oxide synthase in striatal microglia.

THE COMPOSITION OF INTERSTITIAL FLUID IN BEE RETINA: IMPLICATIONS FOR THE SUPPLY OF SUBSTRATES OF ENERGY METABOLISM TO NEURONS.

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Exchanges of small molecules between neurons and glial cells are thought to occur via the interstitial fluid. To understand these exchanges it would be useful to know the composition of interstitial fluid, for which few direct measurements exist. We report that the fluid that rose into a micropipette inserted in the retina of the honey bee drone, a simple, much-studied, piece of nervous tissue, was at least 96% interstitial fluid. This conclusion was deduced from measurements of the concentrations of Na and K in the pipette sample by X-ray microanalysis, and in the interstitial fluid of the intact drone by ion-selective microelectrodes. Comparing these values, and using earlier values for intracellular concentrations, the contamination of the pipette sample by intracellular fluid could be calculated. Concentrations of selected amino acids and carbohydrates in de-proteinated samples were measured by chromatography and compared with published values for retinal homogenates (Tsacopoulos et al, J. Neurosci., 1994). The extracellular concentration of glucose, which is taken up exclusively by the glial cells, was lower than the average intracellular concentration. The extracellular concentration of alanine was more than double the average intracellular concentration and did not change detectably after neuronal metabolism was stimulated by light flashes for 15 min. These results imply that if alanine were a substrate for neurons in drone retina it could be taken up by passive transport, and that the extracellular concentration is regulated.

IMMUNOCYTOCHEMICAL EXAMINATION OF NEURAL PRIMARY CULTURES AND BRAIN SLICES USING ANTIBODIES RAISED AGAINST PYRUVATE CARBOXYLASE

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Pyruvate carboxylase (EC 6.4.1.1.; PC) catalyzes the energy-dependent fixation of carbon dioxide to pyruvate forming oxaloacetate. Mouse monoclonal antibodies and rabbit polyclonal antibodies raised against the liver enzyme were shown to be monospecific as indicated by immunoblotting. Immunocytochemical examination of astroglia-rich primary cultures of rat brain cells revealed a colocalisation of PC with the astroglial marker glial fibrillary acidic protein (GFAP) in many cells. The staining for the enzyme appeared at two levels of intensity. However, there were GFAP positive cells showing no specific staining. Neurons in neuron-rich primary cultures did not stain specifically. In contrast, positive staining for GFAP revealed colocalisation with PC in the 10 % astroglial cells contaminating the neuron culture. In both types of primary cultures only mitochondria were stained by the antibodies thereby emphasizing that PC is a mitochondrial enzyme. This data suggests that PC may be predominantly an astroglial enzyme and that astroglial cells play an important role in the intermediary and the energy metabolism of the brain. These findings are supported by similar results obtained with brain slices and by data of enzymatic activity of PC in these cultures.

RETROGRADE DEGENERATION DIFFERENTIALLY AFFECTS THE PHAGOCYTIC AND IMMUNOLOGICAL FUNCTIONS OF RESIDENT MICROGLIA IN THE RAT OPTIC SYSTEM

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This study was undertaken to investigate the pattern of resident microglial reaction in an experimental model of retrograde retinal ganglion cell degeneration in the albino rat. After unilateral transection of the optic tract at the thalamus, microglial activity along the optic pathway in a group of animals was followed at different times using immuno-cytochemical methods. From 12 days after the lesion, Complement Receptor Type 3 (CR3) positive microglia were observed to increase both in number and in CR3 staining in individual cells in the degenerating optic nerve and retina compared to the contralateral control optic nerve and retina. This pattern persisted till the 22nd day after the lesion, the last time-point in the study. During the same time course, a concurrent and novel expression of Major Histocompatibility Complex Class II molecules (MHC II) was observed in a minority of resident microglia in the degenerating optic nerve. However, MHC II was consistently negative in the degenerating retina at all times examined. Since in the monocyte/ macrophage system CR3 is a specific receptor for the complement degradation product C3bi and is involved in chemotaxis and phagocytosis, and MHC II is involved in the immunological role of antigen-presentation, the different patterns of expression of these two markers in microglia observed in the present experiment suggest that different mechanisms may be involved in their activation. As the retinal optic axons are unmyelinated while the large majority of optic nerve axons are myelinated in the albino rat, myelin degeneration products may be involved in the induction of MHC II expression in resident microglia.

POST-NATAL MATURATION OF THE NEUROMUSCULAR JUNCTION BY SCHWANN CELL .

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Using a monoclonal antibody (6.17) prepared in our laboratory, we have detected at the neuromuscular junction a new molecule which is colocalized with the acetylcholine receptor and which may be synthesized by Schwann cells. We have previously shown that: 1) in vivo in the adult rat this molecule is initially distributed diffusely and concentrates at the synapse 15 days after birth. Its maintenance at the neuromuscular junction is innervation dependent (Koenig et al., *Neurosc. Lett.*, 89, p.265, 1988); 2) during in vitro synaptogenesis, the addition of Schwann cells to neuron-muscle cocultures allows visualization of antigen 6.17 at the synapse and induces synaptic maturation (decrease in number of synapses and appearance of junctional folds). This maturation is inhibited by the presence of antibody 6.17 (Chapron and Koenig, *Neurosc. Lett.*, 106, p.19,.1989). These observations suggest that the Schwann cell may play an antigen 6.17-mediated role in maturation of the neuromuscular synapse.

The present study puts forward two new arguments in favour of this hypothesis: 1) in vitro, synaptic cholinesterase, which is initially embryonic, i.e. heterogeneous (comprising both butyryl- and acetylcholinesterase), develops into the homogeneous adult form (acetylcholinesterase) when Schwann cells are added to neuron-muscle cocultures. This effect is inhibited by the 6.17 antibody; and 2) in vivo, the butyryl- and acetylcholinesterase forms are initially present in equal amounts in the synapse of the newborn, but butyryl-cholinesterase levels diminish sharply on and after the day 15 post-natal, the stage at which the synaptic Schwann cell contacts the muscle fibre (Desaky and Ueharf, 1987).

Finally, we observed that Schwann cells greatly increased the number of AChR clusters in cultured muscle cells, without any involvement of antigen 6.17.

ß-AMYLOID PRECURSOR PROTEIN IMMUNOREACTIVITY IN NORMALLY DEVELOPING AND AXOTOMISED RAT RETINAS

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The ß-amyloid precursor protein (ßAPP), a membrane-associated glycoprotein, is the precursor of amyloid ß/A4 protein, the major component of amyloid in senile plaques in patients with Alzheimer disease. The present study investigates the expression and distribution of ßAPP in developing rat retina, and the effect of neuronal injury on ßAPP by using immunocytochemistry. ßAPP immunoreactivity was expressed transiently from early fetal stages to the first postnatal week by a population of cells which was confined to the inner part of the retina, representing presumably the precursors of ganglion and displaced amacrine cells. ßAPP was also expressed by another population of cells identified as Müller cells, beginning at postnatal day 10 and at all postnatal stages studied. The reaction product, however, was concentrated mainly in the inner part of the radial processes and endfeet of Müller cells. An increase in the ßAPP immunoreactivity was visualised as more strongly reactive and robust radial processes of Müller cells. This was detectable 1-4 weeks after optic nerve lesion. This study provides evidence that ßAPP is expressed in developing retinas by two populations of cells at different times, suggesting that ßAPP may play a role in differentiation and maturation of retinal cells. The increase of ßAPP in Müller cells following degeneration of retinal ganglion cells implies further that cellular interaction may play a role in regulating the synthesis of ßAPP protein. Taken together, our findings suggest that the retina is a useful *in vivo* model system for studying cellular and molecular mechanisms which are involved in regulating the expression or processing of ßAPP.

TRANSITIONS IN PROTEIN EXPRESSION BY RAT PITUITARY INTERMEDIATE LOBE GLIAL-LIKE CELLS DURING ALTERED PHYSIOLOGICAL STATES AND DEVELOPMENT

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Intermediate lobe GFAP immunoreactive (IR) cells possess several protuberances which often branch into secondary and tertiary processes terminating in end-feet. The GFAP cells are distributed in a specific rostro-caudal pattern. The distribution and the numbers of cells differ between males and females. In altered physiological states the glial-like cells stain more intensely and show more and thicker processes. In addition, adrenalectomy and lactation increase, whereas salt-loading decrease the numbers of cells expressing GFAP and abolish the typical pattern of distribution. In contrast, S-100 expressing cells are found to be evenly distributed throughout the lobe and its expression was not affected by the experimental conditions. Double-label immunocytochemistry indicates that GFAP-IR cells are a sub-population of S-100-IR cells. These results suggest that cells expressing S-100 only may be induced to express GFAP under altered physiologic conditions. During the first postnatal days, radially oriented vimentin-IR glial-like cells span the intermediate lobe. During the second postnatal week they change into multi-polar, stellate cells similar to those of the adult GFAP-IR cells and by postnatal day 20 they begin expressing GFAP. These results support the hypothesized glial nature of this cell population.

ION CHANNEL PROPERTIES AND MORPHOLOGY OF GLIAL CELLS IN THE DEVELOPING RAT SPINAL CORD SLICE

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Four types of glial cells were distinguished in the grey matter of rat spinal cord slices at postnatal day 1 to 19 (P1-P19), based on their pattern of membrane currents as revealed with the whole cell patch clamp technique and by their morphological features. The first type of glial cell (A-type) was characterized by symmetrical, non-decaying K^+-selective currents. A-type cells were identified as astrocytes by a positive staining of some cells for GFAP. The second type (B-type) showed symmetrical passive decaying K^+ currents. About half of the B-type cells were positively labelled with a combination of O1 and O4 antibodies, markers of the oligodendrocyte lineage. The third population (C-type) was also positive for the O4 antigen, but these cells were distinct by the presence of inwardly rectifying, delayed outwardly rectifying, and A-type K^+ currents, representing thus most likely "late" glial precursor cells of the oligodendrocyte lineage. The fourth population (D-type) expressed a complex pattern of voltage gated channels, namely Na^+ channels, delayed outwardly rectifying K^+ channels, and A-type K^+ channels. None of the D-type cells could be labelled by oligodendrocyte or astrocyte markers, and are therefore presumably "early" glial precursor cells. The four cell types were found in all regions of the grey matter. When randomly accessing the glial cells, the probability of recording from the presumable glial precursor cells decreased during development. At P1-P3, 69% of the cells showed the precursor cell current pattern (C-type and D-type), while at P13-P15 only 23.5% of the cells revealed this pattern. Concomitantly, the number of astrocytes (A-type) and oligodendrocytes (B-type) increased from 19% and 12% to 41% and 35.5%, respectively.

CHANGES OF CULTURED ASTROCYTE PROLIFERATION RATE INDUCED BY ATP AND RELATED ANALOGUES

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Purines (P), mainly ATP, may act as mitogen agents for different cultured cells (1,2). In astrocytes P modulate cAMP intracellular levels via P_1 type receptors (3) and they stimulate phospholipase C system, intracellular calcium mobilization and eicosanoid release via P_{2Y} sites (4). In this study we evaluated the modification of the proliferation rate induced by stimulation of P_{2Y} receptors and the possible involvement of the eicosanoid pathway, in dissociated primary cultures of rat astrocytes at the 10th day in vitro. Cell proliferation was measured by [^{3}H]-thymidine incorporation during 16 hr and flow cytometric analysis of cell cycle. ATP (5-500 µM) administration to the cultures for 8 hr induced a dose-dependent stimulatory effect on cell proliferation while 16 hr exposure caused a dose-dependent inhibition. The time course of ^{3}H-thymidine incorporation, measured every 2 hr up to 16 hr, showed a significant decrease of astrocyte proliferation during ATP exposure; the withdrawal of ATP after 8 hr allowed astrocytes to recover their proliferative capability, which was enhanced at the 16th hr of observation. HPLC analysis of culture medium samples showed an early and almost complete breakdown of ATP to the respective nucleosides. The activation of A_1 receptors by 5 µM R-phenyl-isopropyladenosine (R-PIA) inhibited astrocyte proliferation; moreover, 100 nM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), an A1 site antagonist, reversed the ATP-induced inhibition of cell proliferation. These findings are in favour of an involvement of A_1 sites in the effect seemingly induced by ATP. The administration to the cultures of 100 µM 2Methylthio-ATP (2MeSATP), a P_{2Y} agonist metabolized as fast as ATP, reproduced effects very similar to the ATP-induced ones. On the other hand, the non hydrolisable ATP analogue, adenosine β- -imidotriphosphate (AMP-PNP), at 100 µM, induced a mitogenic effect as well as the A_2 site stimulation. The inhibition of eicosanoid synthesis achieved by culture exposure to 1 µM dexamethasone or 10 µM mepacrine inhibited the AMP-PNP induced stimulatory effect on astrocyte proliferation. In conclusion, these findings indicate that exogenous ATP, as a consequence of its rapid extracellular break down, exerts a dual influence on astrocyte proliferation by the involvement of both P_1 and P_{2Y} receptors; the mitogenic effect of P_{2Y} receptor stimulation on astrocyte proliferation seems to involve the phospholipase A_2-eicosanoid system.

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PROTEIN TYROSINE KINASE c-YES ASSOCIATES WITH VIMENTIN IN CULTURED MICROGLIA

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Microglial cells are of the mononuclear-phagocyte lineage. Stimulation of their surface receptors leads to a transition from the resting to the active state and the expression of immune properties. Recent findings indicate that signal transduction from antigen-receptor complexes on several types of immunocompetent cells involves increased phosphorylation of tyrosine residues. To understand signal transduction mediated by tyrosine phosphoproteins, it is important to determine which protein tyrosine kinases are present in microglia and whether they are regulated by activation of second messengers. Using antisera to c-yes peptide we have studied intracellular distribution of Yes protein tyrosine kinase, which is present at high levels in neural and hematopoietic tissues. By immunofluorescence confocal microscopy we show that c-yes is present in cultured microglia and that a large proportion of anti-c-yes fluorescence colocalizes with anti-vimentin fluorescence. The specificity of the immunofluorescence was determined by inhibition with c-yes peptide. The extent of association of c-yes with vimentin filaments increases in microglia exposed to dBcAMP and decreases in the presence of phorbol ester TPA. Since our previous studies have shown that under these conditions vimentin in cultured microglia is phosphorylated, these observations suggests that site-specific phosphorylations of the vimentin molecule with protein kinases A and C may regulate its association with the Yes protein tyrosine kinase.

INTERPLAY BETWEEN GENOTYPE AND PHENOTYPE IN GL22 GLIOBLASTOMA CELL LINE

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A tumor develops as a result of genetic alterations which may involve activation of oncogenes or inactivation of tumor suppressor genes, thus subverting normal growth regulatory mechanisms and conferring an in vivo growth advantage to the cells in which they occur. Loss of heterozygosity in chromosome 10 is perhaps the most significant genetic alteration involving tumor suppressor genes and is specifically associated with glioblastoma. Recently we established and characterized a glioblastoma multiforme cell line, GL22, which exhibited loss of both the chromosomes 10 and the presence of unidentified marker chromosomes. The GL22 cells during successive subculturing lost the expression of GFA protein, but maintained the vimentin expression as demonstrated by immunocytochemical, Western and Northern blotting analyses. The finding that GL22 cells consistently express vimentin whose gene is located in the short arm of chromosome 10 led us to reconsider our interpretation of GL22 karyotype, hypothesizing that some unidentified marker chromosomes might carry portions of chromosome 10. This hypothesis was checked by fluorescent in situ hybridization (FISH) with a genomic probe for chromosome 10. Three fluorescent spots were identified in interphase nuclei which seem to correspond to portions of three chromosome markers with different morphology in the metaphase chromosomes. These results are preliminary for studies of both positional gene mapping and tumor biology in the view of understanding the significance of such chromosomal deletions to glial tumor development, assuming that tumor cells would eliminate those genes whose products inhibit their own growth.

RADIAL GLIA: IS IT REGULATED BY ASTROGLIAL AUTOCRINIA?

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Ortogonal organization of the cerebral cortex depends in part on physical scaffolding that radial glia provides to neuronal migration. Processes underlying its induction and maintenance are still largely unknown. Role of astroglial diffusible factor(s) was analyzed in vitro utilizing cerebral cortex(CC)and striatal (S) enriched astroglial subcultures as targets, grown in DMEM/F12 basal medium (BM) alone or conditioned by astroglial subcultures from same regions. Astroglia was characterized by GFAP, vimentin, fibronectin immunocytochemistry, and morphological criteria. Percentage of radial-like (RG), flat (undifferentiated) (UG) and stellate(SG) glia was recorded in CC astroglial subcultures. BM: 14.7-67.6-17.7; CCCM: 78.6-17.9-3.6;SCM: 53.1-32.7-14.3. ANOVA indicated significant differences for incidence of RG in CCCM.Striatal astroglia expressed only UG forms in either culture media. Addition of cAMP or calcitonin gene related peptide to radial-like glia did not result in astrocytic transformation, unless prior dedifferentiation with fetal calf serum was induced, thus suggesting that presence of "radializing" factor(s) interferes with such transformation.
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TRANSITIONAL GLIA AND NONHOMOGENEITIES IN CORTICAL GFAP + ASTROGLIA IN ADULT Cebus Apella MONKEYS

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Radial glia performs a crucial role in cerebral cortex organization during development. Its termination has been linked to astrocytic transformation,possibly related with completion of neuronal migration. Radial- and transforming glia reportedly do not persist into late postnatal life. We report here findings in three adult, Cebus apella monkey brains processed for glial fibrillary acid protein (GFAP), applying the peroxidase-antiperoxidase procedure on cryostat sections. Cortical tissue revealed numerous long astrocytic processes resembling transforming radial glia, stemming from layer I through III/IV in several areas of the temporal, and medial-and dorsolateral frontal cortices. Their distribution was not homogeneous within any given area. These observations indicate a persistent presence of so-called transforming glia in adult New World Cebus apella monkeys, of unknown physiological role, and not observed in adult rats. In addition, a non-homogeneous distribution of cortical GFAP + astrocytes was observed, reinforcing the notion that astroglia plays ubiquitous functions in cortical function.-
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MODULATION OF THE ASTROCYTIC RESPONSE TO A REACTIVE OXYGEN SPECIES GENERATING ENVIRONMENT.

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The effect of exposure of rat astrocytes in primary culture to reactive oxygen species (ROS) generating systems like xanthine/xanthine oxidase and glucose/glucose oxidase has been investigated. Among the glial antioxidant systems tested, a significant increase of the mitochondrial Mn-superoxide dismutase (Mn-SOD) and decrease of the cytosolic Cu,Zn-SOD in response to the ROS generating systems have been observed. In parallel, there is a considerable and rapid decrease of glutamine synthetase activity. Addition of SOD, catalase or mannitol to the cell culture medium during the treatment clearly pointed out that the observed alterations are related to hydrogen peroxide generated by the tested systems. Exposure of the glial cells during 15 h. to hypoxic conditions before treatment prevented the induction of Mn-SOD but did not interfere with Cu,Zn-SOD and glutamine synthetase level modifications induced by the ROS generating systems. These results clearly demonstrate that hypoxia exposed astroglial cells are unable to adapt their antioxidant defence against a burst of ROS , conditions which may occur in brain tissue during reperfusion injury after cerebral ischemia.

Accessory cell function of glioblastoma U373MG

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Accessory cells in the cellular immunesystem are defined by their capacity to stimulate T cells. Furthermore accessory cells were involved in the defense against intracellular pathogens. Within the brain astrocytes may function as accessory cells. As a model we analysed the accessory cell capacity of glioblastoma U373MG.
We found that cells from glioblastoma U373MG express MHC class II antigens and are able to produce cytokines. In addition we were able to show that U373MG cells could activate histocompatible T cells is a MHC class II restricted manner. This capacity of U373MG cells is markedly enhanced after activation with IFNγ.
IFNγ is, besides its immunregulatory function, also involved in the activation of antiparasitic effector mechanisms. An important IFNγ induced effector mechanism directed against intracellular pathogens is the induction of the enzyme indoleamine 2,3-dioxygenase (IDO). We found that IFNγ is able to induce IDO activity within U373MG cells. This IDO activity could be enhanced when cells were stimulated with a combination of IFNγ and TNFα. Activation of U373MG cells with both cytokines result in the induction of toxoplasmostasis. IDO induction and the induction of toxoplasmostasis are linked events as shown by the antagonistic effect of supplemental L-tryptophan on toxoplasmostasis induced by IFNγ and TNFα.
We therefore conclude that glioblastoma cells are able to function as antiparasitic effector cells as well as accessory cell in T cell activation.
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EXPRESSION OF CELL ADHESION MOLECULES AND MHC CLASS I AND II ANTIGENS IN THE NORMAL AND INFLAMED CHOROID PLEXUS AND EPENDYMA

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The choroid plexus has a prominent location at the interface between the central nervous system and the peripheral immune system, and, therefore, is predisposed to early participation in immune reactions. However, its role in the immune response to infectious agents is largely unknown.
To gain further insight into the role of the structures of the ventricular system in meningoencephalitis, we studied the expression of MHC class I and II antigens, intercellular adhesion molecule ICAM-1, and vascular adhesion molecule VCAM-1, which mediate cell adhesion events as well as cellular interactions, in *Toxoplasma* encephalitis (TE).
NMRI mice were orally infected with a low-virulence strain of *Toxoplasma gondii* (DX) and sacrificed in the acute (24 d post infection, p.i.) and chronic stage (180 d p.i.) of meningoencephalitis. The expression of the cell adhesion molecules and the MHC antigens was studied in infected and uninfected animals by light microscopic and ultrastructural immunhistochemistry.
In the normal mouse brain, the epithelium of the choroid plexus and the ependymal cells of the ventricular wall constitutively expressed ICAM-1 and VCAM-1. Both molecules were further upregulated in acute and chronic TE. In contrast, H-2 and I-A antigens were not detectable on these structures in uninfected mice, but were strongly induced in both acute and chronic TE. Ultrastructurally, the immunoreactivity for ICAM-1, VCAM-1 and I-A antigen was located on the surface villi of choroid plexus epithelium and villi of ependymal cells, but not on cilia of ependymal cells.
These data demonstrate a differential expression of cell adhesion molecules and MHC antigens in normal and inflamed mouse ventricular structures. In this property, the choroid plexus differs significantly from cerebral endothelial cells. Our findings may provide a basis for the different capacity of various compartments of the brain to recruit inflammatory cells.

MEMBRANE POTENTIAL-DEPENDENT CLAMP OF INTRACELLULAR pH IN IDENTIFIED LEECH GLIAL CELLS

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The intracellular pH, pH_i, of glial cells in segmental ganglia of the medicinal leech was found to be membrane potential-sensitive due to the activity of an electrogenic Na-HCO_3 cotransporter (1).
We have monitored pH_i with double-barrelled, neutral-carrier, pH-sensitive microelectrodes in exposed, voltage-clamped leech glial cells *in situ*. The relationship between the steady-state pH_i and the membrane holding potential showed a significant dependence with a slope of 110 mV in the presence of CO_2/HCO_3; the slope indicates the membrane potential shift required to change pH_i by one unit. This confirms the stoichiometry of the cotransporter of 2 HCO_3^- : 1 Na^+, and indicates that the equilibrium of the Na-HCO_3 cotransporter *follows* the membrane potential by resetting pH_i.
Lowering the external pH, pH_o, from 7.4 to 7.0 in CO_2/HCO_3 -buffered saline produced a large, partly transient, inward current and a pH_i change from 7.2 to 6.8. This means that pH_i follows pH_o, by more than 80%. The intracellular acidification following the decrease in pH_o could be reversed by depolarizing the membrane, as would be predicted from the thermodynamics of the cotransporter. Thus, any change in the glial membrane potential, as may occur by changes of the K^+ concentration in the extracellular spaces results in an intraglial acidification or alkalinization, and therefore affects pH-sensitive processes in these cells.
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GLIAL CELLS IN HUMAN BRAINS INGEST SERUM PROTEINS FOLLOWING HEMORRHAGE.

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Swollen eosinophilic glial cells are often found in the vicinity of traumatic brain lesions within hours, long before hypertrophy is expected. Experimental studies have shown that astroglia take up serum albumin from edema fluid. We examined human brains to determine whether this form of glial swelling is due to glial ingestion of serum proteins. Twenty-nine human brains removed at autopsy with hemorrhagic lesions hours to 2 months old were examined. The cells in question were identifiable in 25/29 brains. By 9-12 days the swollen eosinophilic cells were difficult to identify because they blended into the neuropil. Fifteen cases were subjected to a battery of histochemical and immuno-histochemical stains. Regardless of the age of the hemorrhage (8 hours to 7 days) or the time delay between tissue death and fixation (1-80 hours, median 12 hours), the cells in question were immunoreactive for IgG, IgM, IgA, and fibrinogen. In the early cases GFAP staining was weak but if the lesions were more than 3 days of age there was intense GFAP immunoreactivity. There was no staining for ubiquitin or for any macrophage markers. Electron microscopy of the cells demonstrated flocculent, membrane-bound material in cells with intermediate filaments. A similar cell response was often seen in the subpial region in patients with meningitis. We conclude that glial cells, largely astroglia, in human brains rapidly ingest serum proteins that have leaked into brain parenchyma. This is another manifestation of the role of astrocytes in regulating the extracellular environment of the brain.

EXPRESSION AND REGULATION OF α AND γ ENOLASE SUBUNITS DURING THE DIFFERENTIATION OF RAT O-2A PROGENITOR CELLS INTO OLIGODENDROCYTES.

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The glycolytic soluble enzyme enolase (2-phospho-D-glycerate hydrolase, EC 4.2.1.11) is found in many animal species and exist as homodimers or heterodimers composed of three distinct subunits α, β and γ encoded by different genes. In the brain of vertebrates, three isomeric forms have been described. The αα enolase (also known as NNE, non-neuronal enolase) is the main form and is expressed in glial cells as well as in undifferentiated neurons. The hybrid form αγ and the homodimer γγ enolase (also known as NSE, neuron-specific enolase) have been regarded as enolase isoenzymes localized in mature neurons **in vivo** and **in vitro**.

Recently, we have shown that glial cells are able to express neuronal markers such as neuromodulin in culture. Here we report the expression of α and γ enolase subunits and of their mRNA during the differentiation of rat O-2A bipotential glial progenitor cells into oligodendrocytes in secondary culture. By using double immunofluorescence staining, we showed that the α subunit is expressed in both O-2A cells (characterized by the A2B5 monoclonal antibody) and mature oligodendrocytes (characterized by a monoclonal antibody against myelin basic protein, MBP). However, the γ subunit is only expressed in mature MBP positive oligodendrocytes. The enzymatic activities of the three enolase isoenzymes were followed during the differentiation of these oligodendroglial cells. High αγ enolase and γγ enolase specific activities were found in the mature oligodendrocytes. Their mRNA levels increased when O-2A cells differentiated into oligodendrocytes. In contrast, no significant changes in αα enolase specific activity, α subunit expression and mRNA levels were observed with time in culture. The basic fibroblast growth factor (bFGF) has been demonstrated to stimulate the proliferation of O-2A progenitor cells and to reversibly block their differentiation in culture. We found that this growth factor delayed the γ subunit expression and inhibited the enzymatic activities of the γγ and αγ isoenzymes in these cells. However, the αα enolase specific activity and the α subunit expression were slightly stimulated by bFGF.

These data indicate that the γ subunit under both αγ and γγ isomeric forms is associated with the mature stage of oligodendrocytes in culture.

UPTAKE OF DIPEPTIDE DERIVATIVES BY ASTROGLIA RICH RAT BRAIN PRIMARY CULTURE

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Transport proteins for dipeptides and oligopeptides are well characterized in small intestine and kidney. A high affinity uptake system for dipeptides was also shown to exist in brain [1,2]. It differs from the other known transporters in several characteristics[2]. ß-Alanine containing dipeptides were used as protease resistant substrates to assay the properties of this transport system.

Taking advantage of the broad substrate specificity we synthesized side chain modified dipeptide derivatives to visualize the cell types involved in dipeptide transport. Uptake of the fluorescent derivative ß-Ala-Lys-N_ε-AMCA was followed by means of spectroscopic and microscopic techniques. ß-Ala-Lys-AMCA, when present at a concentration of 12μM in the incubation medium, was accumulated 30-fold by astroglia-rich rat brain primary cultures. The uptake was shown to be completely blocked by an excess of carnosine (ß-Ala-His). Staining with antibodies against GC and GFAP revealed that the fluorescence-labeled dipeptide is preferentially taken up by type I astrocytes while uptake by oligodendrocytes, neurons and fibroblasts was not observed.

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MÜLLER CELLS IN THE PRIMATE RETINA

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Müller cells and astrocytes comprise the makroglia of the vertebrate retina. Both types of makroglia participate in the formation of the glia limitans of the retina, of the glia limitans of blood vessels, and of the glial wrapping of ganglion cell somata and axons. Until recently, primate retinal makroglia had been studied only sparsely. After having published a quantitative description of retinal astrocytes in the monkey retina (Distler et al., JCN 333:134-147, 1993), we now demonstrate the morphological features of monkey Müller cells. Monoclonal antibodies directed against Müller cells were produced by intrasplenial immunization using cat optic nerve as an antigen. Retinal wholemounts were studied using fluorescence immunohistochemistry and quantitative analysis of the data was performed using high resolution confocal laser scan microscopy. Müller cell morphology in the monkey retina follows the general scheme found in all vertebrate retinae studied so far. The oval-shaped Müller cell somata are located in the inner nuclear layer. From the soma, the upper trunk rises towards the vitread surface usually ending in one fibrillary endfoot. Only occasionally the upper trunks bifurcate into two branches. The vitread endfeet of Müller cells individually wrap ganglion cell somata and axon bundles. Within the inner plexiform layer, numerous horizontal processes arise from the upper trunks of Müller cells. The lower trunk reaches from the soma to the outer nuclear layer where it diverges into numerous rootlets wrapping the somata of photoreceptors. Müller cells are ubiquitous in the monkey retina. There is only a slight density gradient from the parafoveal region to the far periphery. This gradient coincides with subtle morphological differences, with peripheral Müller cells being slightly shorter and thicker than central Müller cells.

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DNA POLYMORPHISMS AT THE HUMAN MYELIN OLIGODENDROCYTE GLYCOPROTEIN (MOG) LOCUS.

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Myelin oligodendrocyte glycoprotein (MOG) is found on the extracellular surface of myelin sheaths and oligodendrocytes, and cell disruption is not necessary for epitopes to be accessible to the immune system. The unique distribution of MOG in the central nervous system (CNS) correlates with the CNS-limited pathology of multiple sclerosis (MS), and therefore the gene coding for MOG is a candidate gene for MS. This gene has been localized on the short arm of chromosome 6, close to the HLA class I region.

We have cloned this gene from a cosmid library. To localize polymorphic markers, we probed the genomic region containing the human MOG locus with synthetic tandemly repeated dinucleotides, and characterized three microsatellites. Using a polymerase chain reaction-based technique, we studied their length variability among healthy individuals. These new polymorphisms provide useful markers for association and linkage studies with MS.

S-100 PROTEIN AND CALPACTIN I BIND TO DIFFERENT SITES ON GFAP AND DIFFERENTLY AFFECT GFAP ASSEMBLY

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S-100 protein, a Ca^{2+}-binding protein of the EF-hand type particularly abundant in glial cells (1), has been shown to bind to GFAP and to inhibits its polymerization into glial filaments (GF) in a Ca^{2+}-dependent manner (2). S-100 protein increases the critical concentration of GFAP assembly, interferes with the GFAP polymer elongation, and disassembles preformed GF (3). In the present study, several members of another family of Ca^{2+}-modulated proteins, the annexin family, were tested as potential regulators of GFAP assembly as investigated by a sedimentation assay. Whereas annexins I, V, and VI proved without effects, annexin II was observed to bind to GF to some extent. Calpactin I, a membrane skeleton protein complex made of two copies of annexin II cross-linked by a dimer of p11, an S-100-like protein, bound to GF to a larger extent than annexin II, without affecting GFAP assembly. On the other hand, p11 neither bound to GF nor affected GFAP assembly. The interaction between calpactin I and GF was dose-dependent with respect to both calpactin I and GFAP. Also, whenever S-100 protein had been included in the incubation mixture, the amount of calpactin I recovered in GF pellets resulted inversely related to the S-100 protein-dependent inhibition of GFAP assembly. By fluorescence spectroscopy, S-100 protein and calpactin I resulted to bind to different sites on GFAP, as judged from the changes induced by individual protein in acrylodan-labelled GFAP. The binding stochiometry was calculated to be 2 mol of GFAP/mol of S-100 protein (dimer), confirming previous data (2), and 1 mol of GFAP/mol of calpactin I. These observations suggest that calpactin I might act to anchor GF at specific membrane sites, and that S-100 protein and calpactin I might regulate the number and topography of GF in proximity of membranes in a concerted manner, on occasion of Ca^{2+}-transients.

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AGE-RELATED SCHWANN CELL HAVE DIFFERENT MITOGENIC RESPONSE TO MITOGENS

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Basic FGF, PDGF-BB, TGFβ and forskolin are mitogens for Schwann cells in serum containing medium and some combinations of these growth factors are mitogens for Schwann cells in serum-free medium. Most previous experiments were carried out on Schwann cells from 2-5d old rats. It is not clear whether Schwann cells of different postnatal ages have different mitogenic responses to the mitogens.

In order to elucidate this question, 1d, 4d and adult rat Schwann cells have been dissociated from the sciatic nerve and exposed to different factors(including bFGF, PDGF-AA, PDGF-BB, TGFβ and forskolin) and their combinations. Our results showed that Schwann cells at different postnatal ages had different responses to mitogens in serum-free medium. bFGF and forskolin alone were mitogens for adult Schwann cells, but not for younger cells. Schwann cells from the adult rat expressed a higher rate of DNA synthesis than younger Schwann cells in response to the same growth factors. Adult Schwann cells in culture required less exogenous cAMP or cAMP elevators in response to mitogens. A high dose of forskolin (20μM) or dbcAMP($x10^{-3}$M) gave a high rate of DNA synthesis in 1 day Schwann cells but inhibited that of adult Schwann cells. TGFβ and TGFβ coupled with forskolin were not mitogens for Schwann cells at any age The combination of TGFβ and bFGF was weak mitogen for 4 day and adult Schwann cells but not for 1 day Schwann cells. TGFβ coupled with both bFGF and forskolin was a strong mitogen for 1 day Schwann cells. The mitogenic potency of this combination decreased with age.

THE BASAL LAMINA CAN STIMULATE A DIFFERENTIATION OF MIGRATED SCHWANN CELLS IN THE PERIPHERAL NERVOUS SYSTEM OF THE ADULT RAT

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Freezing injury of the glabrous toe skin in the adult rats results in destruction and removing of all cellular components of the skin sensory corpuscles. Their basal lamina and other extracellular matrix components seems to be intact. The migration of Schwann cells from the distal stump of the homolateral transected sciatic nerve was observed into the space of former corpuscles. The Schwann cells changed their shapes to the terminal Schwann cells and occupied the narrow circular spaces limited by the basal lamina. This suggests that the basal lamina stimulates the differentiation of the migrated Schwann cells to the terminal Schwann cells without presence of the sensory axon. Schwann cells were also able to migrate into the dorsal root ganglia of the adult rats following their *in situ* freezing injury. Four weeks after freezing treatment the migrated Schwann cells took up the ring-shaped position, which is typical for the satellite cells, in absence of the primary sensory neurons. On the basis of our results we can suggest that during ontogenetic development the basal lamina of the peripheral glial cells is enriched by molecules that are capable to stimulate a differentiation of the migrated Schwann cells in the adult rats.

ASTROGLIOSIS FOLLOWING bFGF, PDGF OR EGF MICROINJECTION IN THE INTACT OR INJURED BRAIN OF ADULT RAT.

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Basic fibroblast growth factor (bFGF) is a polypeptide known for its trophic and mitogenic properties. Sensenbrenner and co-workers have shown that bFGF stimulates **in vitro** the proliferation and maturation of astroblasts. **In vivo**, we have shown that a micro-injection of bFGF in the cortex or in other brain structures resulted in the appearance of an astrogliosis with an increase of the number of astrocytes and of the labeling index. When bFGF was injected in the site of an electrolytic lesion made in the cortex, the number of astrocytes was not strongly increased but the pattern of gliosis was accelerated. Two other growth factors, PDGF and EGF which are expressed in adult brain have also been shown to stimulate **in vitro** the proliferation of astroblasts. The present study was designated to know 1) whether a microinjection of PDGF or EGF made in an intact brain would also result in a reactive gliosis and 2) whether PDGF or EGF injected following an electrolytic lesion in the brain would result in an increase of the number of astrocytes. Twenty two-month-old Wistar rats were used for this study and were sacrified after various post-operative delays. The brains were taken and glial fibrillary acidic protein (GFAP) was revealed by immunohistochemistry. We found that PDGF or EGF injected in the intact brain result in the appearance of an astrogliosis. With PDGF, the astrocytes are very big with thick processes while with EGF, the astrocytes are small round cells with long slender processes. When these two factors are injected in the lesion site, the morphology as well as the number of astrocytes are not changed compared to the lesion-induced gliosis. These results suggest that PDGF and EGF are involved like bFGF in the control of the astrogliosis elicited by brain injury.

PROPERTIES OF POTASSIUM CURRENTS OF MICROGLIAL CELLS UNDER DIFFERENT CULTURE CONDITIONS

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Cultured microglial cells from murine brain were stimulated by the addition of macrophage-colony stimulating factors (M-CSF) and granulocyte/macrophage-colony stimulating factors (GM-CSF), respectively. These cytokines induce distinct functional states which are well characterized by immunological methods. We investigated voltage gated potassium currents of both different cell types. Currents were recorded using the whole cell patch clamp technique. After establishing the whole cell configuration, cells were held at -60 mV. 200 ms hyperpolarizing and depolarizing voltage commands were applied in 10 mV steps between -150 mV and +30 mV. In most cells derived from M-CSF-driven culture only inward potassium currents could be observed. In contrast, in most GM-CSF-grown cells outward potassium currents could be recorded. A small part of cells under investigation did not show any active voltage gated currents at all. Currents evoked by hyperpolarizing commands showed properties of inward rectifying potassium currents. These currents activated negative to the potassium equilibrium potential and could be blocked by extracellular applied barium and cesium. Outward currents activated at depolarizing potentials of about -30 mV. These currents were blocked by extracellularly applied 4-AP at millimolar concentrations. They were reduced only to a small extent by application of 10 mM barium or TEA (10-20 mM).

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CHARACTERIZATION OF ASTROCYTIC LAMELLAE BY MORPHOMETRICAL AND FRACTAL PARAMETERS

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Lamellae are flat sheet-like protrusions of an astrocytic soma and its processes. They do not contain organelles and have a thickness of about 0.2 µm in cross sections. To visualize lamellae, single astrocytes were intracellularly stained with horseradish peroxidase. Light and electron microscopical (EM) photographs of these homogeneously filled astrocytes were used. for morphometrical analysis.

Results of first order mathematical calculations discern two subterritories belonging to one astrocytic cell territory: an "autocontrol space" occupied by cell soma, processes and lamellae of one cell alone, and an "interdigitation space" where overlapping of processes and lamellae to those of neighbouring astrocytes should occur.

To further support the "two-space-hypothesis" of an astrocyte fractal dimensions of lamellar profiles were measured by image analysis. For this purpose a program was developed to first seperate lamellae from stained processes on drawings from EM-photographs. Using different techniques (e.g. dilation, box counting) a significantly higher fractal dimension (D~1.4) was found for the **lamellar pattern** of the autocontrol space compared to the interdigitation space (D~1.1). According to our preliminary results this can not be explained by different fractal properties of **single profiles** but is based on the properties of the lamellar profile distribution pattern.

NGF INCREASES $[Ca^{2+}]_i$ IN REGENERATING MATURE OLIGODENDROGLIAL CELLS

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Recently, we have shown that Nerve Growth Factor (NGF) has profound effects on process formation and proliferation in mature pig oligodendroglial cells. The signal transduction mechanism subserving this effect is not understood. Changes in the intracellular free Ca^{2+} concentration, $[Ca^{2+}]_i$, have been proposed as a possible event in the NGF induced signal transduction cascade. We therefore investigated the action of NGF on $[Ca^{2+}]_i$ by measuring fluo-3 fluorescence. NGF caused transient increases in $[Ca^{2+}]_i$, which could be inhibited by anti-NGF antibody. The rise in $[Ca^{2+}]_i$ was in part due to influx of extracellular Ca^{2+} since it was markedly attenuated in Ca^{2+} free medium. It also depended on release of Ca^{2+} from intracellular Ca^{2+} stores as tested by prior depletion with cyclopiazonic acid. We are now characterizing the properties of the intracellular Ca^{2+} stores.

OXYTOCIN-BINDING SITES IN RAT SPINAL CORD CULTURED ASTROCYTES

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Beside its peripheric hormonal effects, oxytocin (OT) could act as a neuromodulator in the central nervous system. Indeed, local OT applications can modulate the electrical activity of hypothalamic and spinal cord neurones, but also induce astroglial plasticity within the hypothalamo-neurohypophyseal system. As OT-binding sites were detected by binding studies on hypothalamic cultured astrocytes and by autoradiography on spinal cord tissue sections, detection and pharmacological characterization of OT-binding sites were attempted on rat spinal cord cultured astrocytes. The binding studies were performed with a highly selective iodinated OT antagonist ([^{125}I]OTA) on 12-day-old cultures. By combinaison of autoradiography and immunocytochemistry, OT-binding sites were observed on cells labelled with GFAP (Glial Fibrillary Acidic Protein), a specific astroglial marker. Saturation studies suggested the presence of a single class of binding sites of high affinity for the OT antagonist (K_D=0.038 nM), as already described in the rat at the central level, at the periphery and in hypothalamic cultured astrocytes. The competition studies indicated that OT displaced the labelled ligand with a high affinity of 1 nM, as already described, whereas vasopressin had a much lower affinity (20 nM) which is in contrast with the affinity reported for hypothalamic astrocytes. This feature may suggest that OT-binding sites detected on spinal astrocytes have pharmacological characteristics which are different from those reported for hypothalamic cultured astrocytes.

COMPARATIVE CHARACTERISTICS OF NH_4^+ AND K^+-INDUCED [^{3}H]TAURINE (TAU) RELEASE FROM CULTURED MÜLLER GLIA

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Low, pathologically relevant concentrations of NH_4^+ (0.5-5 mM) release TAU from Müller cells by a mechanism which partly differs from cell swelling-related release by high (65 mM) K^+ (1). In this study we compared the effects of the two stimuli with regard to their sensitivity to: protein kinases inhibitors (PK-I), 24 h treatment with 1 mM ammonia (AMT), and pH and ion transport modulators. The cAMP-dependent PK-I - HA 1004 completely abolished NH_4^+-induced release but only partly K^+-induced release, indicating a greater concordance of the former with the receptor-stimulated TAU release mediated by cAMP which is thought to be triggered by conformational changes of cytoskeleton proteins (2). PKC-I - H7 and AMT, both causing TAU leakage, abolished stimulation of TAU release by K^+ but enhanced that by NH_4^+. A pHi reducing agent (sodium propionate), Na^+/H^+-exchange blocker (amiloride) and a calcium ionophore (ionomycin), each produced TAU leakage and inhibited more significantly K^+-dependent than NH_4^+-dependent TAU release. The results indicate that well preserved cell membrane integrity appears much more critical for K^+- than NH_4^+-dependent TAU release and that different pools of TAU may be involved in either case.

1. L. Faff-Michalak et al. (1994) Glia (in press)
2. D. L. Martin and W. Shain (1993) Neurochem. Res. 18:437.

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GFAP GENE EXPRESSION DURING POSTNATAL DEVELOPMENT

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During astroglial maturation, most of the GFAP-mRNA transcripts appear during the early perinatal period. This early transcription is associated with a rapid turnover of the encoded protein (half-life=16-18 hours). At that time, GFAP appeared as a polymerized network close to the cell nuclei and the centriolar region. GFAP-mRNA steady-state levels,measured by Northern-blot,increase during the proliferating state. But while astrocytes change their shape and stabilyze their processes,the message level decreased. This diminushion was correlated with the drop of the amount of transcripts and consistant with a transcriptional control during this period. A stabilization of the GFAP is supported by a low turnover rate(half-life= 5-6 days). During that time, astrocytes develop numerous processes,in which both GFAP and GFAP-mRNA are expressed. A role of GFAP in the early mitotic control may be hypothesyzed . GFAP is at minima involved in the stabilization of the astroglial cell-shape in a given environment. Changes in GFAP expression in physiopathological conditions, may modify signalling transduction and related events and also cell to cell interactions. An antisens GFAP-mRNA strategy is under investigation in order to understand better the role of this protein during the cell division and diffe= rentiation.

Purification of a neural proteoglycan with neurite outgrowth promoting properties

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Glial cells play an important role in the regulation of neural pattern formation during neurohistogenesis. In order to obtain further insight into the molecular basis of neuron-glia interactions, we have characterized the chondroitin sulfate proteoglycan DSD-1-PG identified by the monoclonal antibody (MAb) 473 (Faissner, Soc. Neurosci. Abstr. 14, 920 (1988), Faissner et al., Soc. Neurosci. Abstr. 19, 435 (1993)). DSD-1-PG is selectively expressed on the surface of immature astrocytes and oligodendrocytes, but not detectable on the surface of neurons or fibroblastoid cells. DSD-1-PG was enriched from detergent-free extracts of postnatal mouse brains by immunoaffinity chromatography and was further purified by ion-exchange chromatography. Purified DSD-1-PG displays an apparent molecular weight of 800-1000 kD in size exclusion chromatography or SDS-PAGE and a major core glycoprotein of Mr 350-400 kD after digestion with chondroitinase ABC. Specific polyclonal rabbit antibodies recognized the same molecule as MAb 473 according to Western blots, immunolocalization experiments and sequential immunoprecipitation studies, consistent with the notion that MAb 473 recognizes one singular PG. The DSD-1-epitope proved sensitive to chondroitinase ABC, but resistant to chondroitinase ACII, suggesting that MAb 473 reacts with a chondroitin/dermatan sulfate hybrid chain. In order to investigate its functional properties, embryonic day 18 rat hippocampal neurons were grown on DSD-1-PG adsorbed to poly-ornithine. Under these conditions, DSD-1-PG promoted neurite outgrowth, an effect which could be blocked by MAb 473 and by enzymatic removal of the glycosaminoglycan chain(s). We propose that DSD-1-PG might be one of the components of immature glial cell surfaces favouring neurite extension.
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α6, β4 AND β1 INTEGRINS ARE DIFFERENTIALLY EXPRESSED DURING PERIPHERAL NERVE DEVELOPMENT, REGENERATION, AND IN MYELINATING VERSUS NON-MYELINATING SCHWANN CELLS.

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Schwann cell differentiation and myelination are influenced by interactions with basal lamina. Integrins are adhesive receptors that can mediate cell-extracellular matrix interactions. Since Schwann cells highly express α6β1 and α6β4 integrins, we investigated the pattern of expression of α6, β4 and β1 integrin subunits during peripheral nerve development and regeneration.

The expression of β4 mRNA and protein, but not of α6 and β1 mRNA, is developmentally regulated in peripheral nerve. Similarly, the expression of β4, but not of α6 and β1, in Schwann cells depends on the presence of axons, and this modulation is transcriptionally mediated. Furthermore, the expression of β4, more than α6 mRNA is induced in Schwann cells cultured in the presence of forskolin, a substance that induces the expression of other axonally-dependent genes in Schwann cells. Finally, in myelinating Schwann cells, α6 and β4 co-localize at the ab-axonal surface, which interfaces with their basal laminae. In contrast, in non-myelinating Schwann cells, α6 and β4 are expressed at lower levels, without an organized pattern.

Since β4 dimerizes more avidly with α6 than does β1, the induction of β4 expression during peripheral nerve development may change the profile of integrin expression with important functional effects in Schwann cells. Also, the timing and location of β4 expression, taken together with the possiblity that myelinating Schwann cell basal laminae may contain the ligand for α6β4, suggest that α6β4 may play an important role in myelination.

COLCHICINE-INDUCED REACTIONS IN FASCIA DENTATA

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Intradentate injections of colchicine induce a prompt deterioation of dentate granule cells. This study focus on the glial and macrophage reactions to colchicine-induced degeneration of dentate granule cells and the mechanism of action of locally applied colchicine.

Adult male Wistar rats were injected with colchicine (0.06 µl, 7 mg/ml) into 4 different septodorsal levels of fascia dentata. The animals were perfused from 12 h to 42 d after with buffered formalin. Thereafter the brains were sliced into 50 µm thick vibratome sections, and the sections stained with toluidine blue and markers for astro- and microglial cells and macrophages. Additional rats received intradentate injections with the colchicine-derivative ß-lumicolchicine, which exerts the non-tubulin, but *not* the tubulin effects of colchicine. Finally mixed primary glial cultures were exposed to colchicine and ß-lumicolchicine.

The analysis showed, that the glial response to colchicine-induced degeneration of dentate granule cells has its own characteristics, with -1) an early and extensive recruitment of ED1, LCA and vimentin reactive blood-derived macrophages, -2) a transient transformation of OX42 reactive microglial cells in all hippocampal subregions into ameboid-like cells, and -3) a transient occurrence of globoid, dividing micro- and astroglial cells at day 2 and 4, respectively - in addition to the expected pattern of typical degeneration-induced glial and macrophage reactions in fascia dentata. The profound effect of colchicine on the microglial morphology was confirmed in *in vitro* studies, where ramified microglial cells retracted their processes and rounded up following exposure to colchicine. This effect was due to the binding of colchicine to tubulin proteins, as ß-lumicolchicine had no such effect on the microglial cells.

We conclude, that colchicine: -1) has a direct tubulin-related effect on microglial cell morphology, and -2) amplifies the visible number of lesion-induced micro- and astroglial mitosis. We finally suggest, that this model may be usefull in mechanistic studies of leukocyte migration in the brain.

CYTOKINE-DEPENDENT FUNCTIONAL STATES OF CULTURED MICROGLIA CORRELATE WITH THEIR ELECTROPHYSIOLOGICAL BEHAVIOUR

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To investigate the effects of the two macrophage/microglial growth factors astrocytes are able to produce, in vitro-differention of microglia was stimulated by the addition of M-CSF or GM-CSF during primary murine brain cell culture. As demonstrated by analysing the proliferative response and expression of MAC-1 and F4/80 markers, both factors obviously do not promote the development of microglial cells with biased growth requirement and phenotype. However, these cytokines proved to induce distinct functional states when comparing the cells with respect to (i) their electrophysiological properties; (ii) the secretory and cellular immune functions and (iii) their antiparasitic activity.

On most cells derived from M-CSF-driven culture only an inward rectifying K^+ current could be observed. On GM-CSF-grown cells we measured both inward and outward K^+ currents evoked by hyperpolarizing and depolarizing voltage steps. The functional particularity of these microglial cells is reflected in an instrinsic capability for excellent antigen presentation to T_H1 and T_H2 clones and is paralleled by an enhanced delivery of the relevant costimulatory signals: IL12 and membrane-IL1, respectively. Upon gradual stimulation by LPS microglia developed with M-CSF released more IL1 and TNFα activity, those cultivated with GM-CSF proved superior in IL6 secretion. When infected with virulent toxoplasms, only GM-CSF-differentiated cells were able to restrict spontaneously the intracellular parasite multiplication via formation of NO. M-CSF-grown cells, on the contrary, required prior IFNγ-activation to efficiently perform antigen presentation and resistance against *Toxoplasma gondii*.

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IN VITRO AND *IN VIVO* STUDIES OF OLFACTORY NERVE ENSHEATHING CELLS.

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The olfactory bulb of the adult rat is the only central nervous system tissue (CNS) that can support the growth of neurons throughout the life of the animal. It is thought that this property is due in part to the glial cells that reside in the outer layer of the olfactory bulb, termed olfactory nerve ensheathing cells (ONECs). To characterise the cellular and molecular properties of these cells in more detail, we have devised a method for purifying them from the olfactory bulb using the O4 antibody and fluorescence activated cell sorting (FACS)[1]. Using a panel of glial markers we have shown that the antigenic profile of the sorted ONECs changes with time in culture. In serum free medium (SATO) the entire cell population lost O4 expression and gained NGF receptor and GFAP expression. A subset of cells expressed high levels of fibrous GFAP, while E-NCAM expression varied slightly, detectable in half of the cell population from day 1 in culture. When cells were grown in astrocyte conditioned medium (ACM) or 10% FCS, both potent mitogens, the rate of these changes increased and E-NCAM expression was lost entirely. In 10% FCS, expression of GFAP and NGFr was reduced. When comparisons were made with the immunolabelling of tissue sections of the olfactory bulb, it was found that these antigenic changes did not correlate with development although certain similarities became apparent. With time in culture the sorted ONECs developed into two antigenically and morphologically distinct cell types; i) GFAP+/E-NCAM+/NGFr± fibroblast-like cells resembling type-1 astrocytes and ii) GFAP±/E-NCAM-/NGFr+ process-bearing cells resembling non myelinating Schwann cells. This was less apparent in the presence of serum. Interestingly, analysis of the antigenic profile of the developing olfactory system also suggested that these two cell types exist *in vivo*. Experiments are in progress to determine if these two cell types are related.

[1]Barnett, S.C. Hutchins, A-M and Noble, M. (1993) Purification of olfactory nerve ensheathing cells from the olfactory bulb. Dev. Biol. 155:337-350.

EFFECT OF INTRACELLULAR K^+ SUBSTITUTION AND ADDITION OF INTRACELLULAR Cs^+ ON GUINEA PIG MÜLLER GLIAL CELLS IN VOLTAGE CLAMP EXPERIMENTS

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The cell membrane of most glial cells possesses a very low input resistance due to their high K^+ conductance. This feature is unfavourable if one attempts to characterize membrane currents not carried by K^+ in this type of cells. For this reason we tried to block completely the K^+ currents of Müller cells. In whole-cell voltage-clamp experiments using suction electrodes for recording the intracellular K^+ was completely replaced by NMDG (100 mM) and Cs^+ (30 mM). However, amplitudes of outward currents evoked by depolarizing voltage steps from a holding potential of -80 mV did not markedly differ from those recorded under control conditions with 130 mM K^+ inside the cell. When 1 mM Ba^{2+} was added to the extracellular solution or K^+ was removed, the amplitude of outward currents elicited by repetitively applied depolarizing voltage steps diminished continuously until they disappeared. Hence, it can be concluded that despite replacement of intracellular K^+ this ion accumulates inside the cell due to K^+ inward currents caused by the negative holding potential. If this influx is stopped, as by extracellular addition of Ba^{2+} or removal of K^+, intracellular K^+ accumulation ceased.
Interestingly, the outward K^+ currents were not strongly blocked by internal Cs^+ (30 mM), an effect that could be observed also when the internal solution contained 100 mM K^+ and 30 mM Cs^+. Thus, Müller cells seem to express a K^+ channel that is relatively insensitive to blockade by Cs^+.

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IMMUNOCYTOCHEMICAL CHARACTERIZATION OF NON-NEURONAL CELLS IN THE RAT OPTIC NERVE AFTER CRUSH INJURY

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After rat optic nerve crush, a reduction of glial fibrillary acidic protein (GFAP) immunoreactivity and the invasion of phagocyting cells have been described. In order to characterize this cellular responses in more details we used antibodies against the astroglial proteins GFAP, S100 and Vimentin. Anti-Vimentin was also used as a marker for microglial and other phagocyting cells as well as ED1 antibody and anti-Lysozym. Investigations were performed 7 hours, 2, 6, 8, 14 and 21 days post lesion (dpl), both at light microscopical (ABC-method) and electronmicroscopical level (postembedding immunogold-method). We showed that astrocytes and their processes repopulate the lesion site beginning with 6 dpl, demonstrated by immunogold-doublelabelling experiments, which showed the codistribution of S100P/Vimentin and GFAP/Vimentin in the same cells. S100P was never and Vimentin was only very rarely detected in other cells than astrocytes. Therefore, the overall majority of phagocyting cells was completely lack of Vimentin. In contrast, phagocyting cells were labelled in the lesion site in considerable numbers with anti-Lysozym and anti-ED1 from 2 dpl onwards, increasing in number until 8 dpl and decreasing in number thereafter. We consider these cells as blood-born macrophages, not as microglia, which seems to play a role of minor importance in this injury model. According to our knowledge, ED1 antibody was performed for the first time in postembedding immunogold labelling experiments; we showed that at subcellular level the phagocytic vacuoles were labelled depending on their state of activation.
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THE BEHAVIOUR OF RAT 0-2A PROGENITOR CELL LINES FOLLOWING TRANSPLANTATION INTO GLIA-FREE CNS LESIONS

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This study is a continuation of early attempts to generate large numbers of 0-2A progenitors for transplantation into CNS lesions using either retroviral immortalisation (Trotter et al., 1993, *Glia* 9: 25-40) or growth factor-mediated expansion 65(Groves et al., 1993, *Nature* 362: 453-455). Here we describe the creation of an 0-2A progenitor cell line in which these two approaches were combined. The cooperative interaction between PDGF and bFGF was used to optimise retroviral incorporation of a temperature sensitive SV40 large T gene into 0-2A progenitors, and, post-infection, to maintain a stable 0-2A phenotype within the cell line. At the non-permissive temperature, these cells behaved in a similar fashion to 0-2A progenitors from primary culture in terms of their differentiation potential and in their growth factor responses. Following transplantation into glia-free areas in the adult rat spinal cord, a proportion of cells differentiated into myelin-forming oligodendrocytes, while a smaller proportion differentiated into astrocytes, thereby illustrating that the bipotentially of 0-2A progenitors *in vitro* can also be demonstrated *in vivo* . These results are compared with the behavior of a second O-2A progenitor cell line, CG-4, labelled by transfection with the *lacZ* reporter gene, following transplantation into similar glia-free areas of rat spinal cord.

Tenascin expression in denervated peripheral nerves of wild type and Ola mutant mice: possible implications for axonal regeneration.

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The present study focuses on the up regulation of tenascin during Wallerian degeneration in the C57 BL/6J mouse and a mutant (C57 Bl/Ola, briefly termed "Ola mouse") which is characterized by an age dependent delay of Wallerian degeneration. In the wild type mouse we found that non-myelinating Schwann cells start to express tenascin 2 days after lesion whereas myelinating Schwann cells first express tenascin 5 days after lesion. In the mutant (Ola mouse) neither myelinating nor non-myelinating Schwann cells expressed tenascin for up to two weeks post lesion in animals younger than 8 weeks. In older Ola mice (16 weeks) we could detect tenascin 5 days post lesion in a muscle nerve (the quadriceps nerve branch of the femoral nerve) but not in a cutaneous nerve (the saphenous nerve branch of the femoral nerve). Further investigations (assessment of the disappearance of neurofilament immuno-reactivity and the disorganisation of axonal profiles and myelin sheaths by electron microscopy) revealed that myelinated motor axons in the Ola mouse muscle nerve degenerate at almost normal speed. Anterograde tracing studies showed regenerating axons in the muscle branch (associated with degenerating Schwann cells) but not in the cutaneous branch. These findings confirm the hypothesis that Wallerian degeneration is an important prerequisite for successful regeneration in the peripheral nervous system.

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DISREGULATION OF HUMAN PNS MYELIN PROTEIN EXPRESSION IN NEUROPATHIES ASSOCIATED WITH ANTI-MAG ANTIBODIES.

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The L2/HNK-1 carbohydrate structure is present on glycolipids and on a number of glycoproteins of the nervous system that are involved in cellular recognition and/or adhesion. Most patients with demyelinating paraproteinaemic polyneuropathy (PPN) have monoclonal IgM autoantibodies (M-IgM) that react with the L2/HNK-1 determinant of MAG and P_O. Clinical and experimental studies have suggested a causal role of these anti-MAG antibodies for the development of the neuropathy. As part of our investigation of myelin gene expression in PPN patients, we plan to test the hypothesis that anti-MAG M-IgM autoantibodies may alter the expression of HNK-1 bearing myelin proteins such as MAG, P_O or PMP22. Preliminary results of immunohistological analysis of sural nerve biopsies from patients with PPN and anti-MAG M-IgM gammopathy were obtained by using monoclonal antibodies to myelin proteins : expression of the myelin proteins MBP, MAG and Po and the phospholipid GalC seems to be decreased in a comparable amount to the myelin loss; however in the remaining myelin the detectable content of MAG seems decreased in some of the myelinated fibers, while P_O, MBP, GalC and S100 protein are unchanged. In contrast, GFAP protein expression appears to be upregulated in these patients. In conclusion, circulating anti-MAG M-IgM in the serum of PPN patients may deregulate the expression of MAG itself as well as some L2/HNK-1 negative proteins.

EFFECTS OF TGFβ1 ON CULTURED ASTROCYTES.

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We first have shown the effects of transforming growth factor (TGFβ1) on cell proliferation and differentiation, extracellular matrix components and cytoskeletal structures in astrocytes from primary cultures of cerebral hemispheres or cerebellum and from an immortalized cell line (J. Neurochem. 1990, 54, 1056-1061; Exp. Cell. Res. 1992, 202, 316-325; Glia 1993, 7, 193-202). TGFβ1 inhibited cell growth and decreased the induction by hydrocortisone of glutamine synthetase, an astrocyte-specific enzyme. It stimulated the synthesis of matrix components and their integration into a network. The composition of extracellular matrix was altered by a such treatment, in particular in the cell line.

Concomitantly, cells treated by TGFβ1 became enlarged, spread onto the substratum, and showed a modified cytoskeleton. Glial fibrillary acidic protein (GFAP) presented an important network of numerous fibrils dispersed into the cytoplasm. After one day of treatment, the content of actin was increased and the cells displayed well-developed bundles of actin fibrils organized in stress fibers. Actin reorganization started from one hour whereas actin synthesis was not still stimulated. An increase in the number of lamellipodia accompanied stress fibers appearance. The kinetic of changes in actin cytoskeleton and its organization were distinguishable from those promoted by serum, PDGF and endothelin.

2KB SEQUENCE OF THE GFAP GENE PROMOTER ARE ACTIVATED IN REACTIVE ASTROCYTES AFTER BRAIN INJURY, IN TRANSGENIC MICE

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The expression of the glial fibrillary acidic protein (GFAP), a component of astroglial intermediate filaments, is regulated under developmental and pathological conditions. In order to characterize DNA sequences involved in such regulations, we produced transgenic mice bearing 2 kb of the 5' flanking region of the murine GFAP gene linked to the *Escherichia coli* β-galactosidase (β-gal) reporter gene. Seven transgenic lines were obtained. We demonstrated that the regulatory elements present in the transgene GFAP-*spretus*-nls-LacZ are sufficient to direct transgene expression in the neural tissue and to target *in vivo* the expected localization of GFAP to where it is normally present. In the developing brain, β-gal activity and GFAP appeared simultaneously and in the same region, on embryonic day 18 (ED 18), suggesting that the 2 kb of the promoter contains the regulatory sequences responsible for the perinatal vimentin/GFAP switch. In addition, we demonstrated that the 2 kb sequence of the GFAP promoter used in the transgene possess elements which are activated after a surgical injury, thus permitting to study some aspects of reactive gliosis in these transgenic mice.

VOLUME REGULATION AND ION HOMEOSTASIS IN THE HUMAN GLIOBLASTOMA U373 MG CELL LINE

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Cerebral ischemic conditions are often associated with an impairment of the volume regulation in the glial cell compartment. We studied volume regulation and ion homeostasis in the human glioblastoma cell line U373 MG. Intracellular pH was measured by ratio imaging of BCECF fluorescence, whereas the fluorescent probe SBFI was used to study dynamics of the intracellular sodium concentration. In addition, cell volume was quantified by digital image analysis.

Upon addition of 20 mM Na-propionate, an immediate intracellular acidosis was observed, together with an increase in free Na^+_i. Within five minutes an increase of the cell diameter of 12±2.1 % (mean ± SEM, n=60 cells) was found. This increase was inhibited in the presence of 10^{-5} M EIPA, a specific Na^+/H exchange blocker (-1.2±1.2 %) and also when extracellular sodium is substituted by the membrane-impermeable choline (-0.9±1.4 %). Time-dependent analyse showed that the cell diameter increased to a peak of 20±2.5 % after 2 minutes, followed by a reactive volume decrease within 15 min..

Ischemic conditions were simulated by incubating the cells with an ischemic medium, consisting of 125 mM Na^+, 12 mM K^+, 10 mM Hepes, 0.5 mM MgCl2, 0.02 mM deoxyglucose, 20 mM lactate, and 1.5 mM NaCN at a pH of 6.5. Incubation of the cells with this medium caused again a biphasic volume response. An increase in cell diameter (14.2±4.1 %) up to 2 minutes, was followed by a reactive volume decrease back to basal levels. This change in diameter was also prevented by 10^{-5} M EIPA.

The data suggest that this cell line has a EIPA-sensitive functional Na^+/H^+ exchanger and may be useful in studying Na^+-dependent volume regulation of the glial compartment in ischemic conditions.

CONTRIBUTION OF MBP+ AND PLP+ CELLS FROM NORMAL AND JIMPY BRAIN TO GENERATE THE OLIGODENDROCYTE CELL LINE IN CULTURE

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The relationship between oligodendrocyte proliferation and the expression of myelin basic protein (MBP) and proteolipid protein (PLP) has been carefully investigated. Mixed primary glial cultures derived from newborn rat and mouse brain hemispheres at 10, 17 and 27 day *in vitro* (DIV) and oligodendrocyte-enriched cultures obtained from 10-12 DIV normal and jimpy mouse primary glial cultures were used.

Oligodendrocytes in S phase of the cell cycle were detected by immunostaining for BrdU after 1-2 h pulse. Multiple detections of MBP and PLP by immunolabeling and their respective mRNAs by *in situ* hybridization both combined with BrdU immunocytochemistry were performed.

At 10 DIV in primary cultures, 3% of MBP^+cells were MBP^+BrdU^+. Similar number was obtained for PLP and for MBP and PLP mRNAs.

In oligodendrocyte secondary cultures at 2 and 4 DIV, 9-10% of PLP^+ or MBP^+cells were dividing. In addition, 4-5% of MBP^+cells were observed in a mitotic figure in oligodendrocyte cultures obtained after isolation of glial cells by Percoll gradient .

The number of MBP^+BrdU^+cells in normal oligodendrocyte cultures was increased by 2-3 fold after treatement with bFGF, while no significant increase was observed in the number of MBP^+BrdU^+ jimpy oligodendrocytes after similar treatement.

Our data showed (1) that the presence of astrocytes in primary cultures reduced the proliferation rate of oligodendrocytes, (2) a defect in jimpy oligodendrocytes to respond to bFGF treatment and (3) the contribution of dividing $MBP^+$and PLP^+cells to generate oligodendrocytes.

GLIOGENESIS IN THE ADULT PERIPHERAL NERVOUS SYSTEM OF *DROSOPHILA MELANOGASTER*

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In the fly wing, glial cells are present along peripheral nerves and wrap them with their cytoplasmic processes. In contrast to the stereotyped arrangement shown by the wing sensory neurons, glial cells display a variable organization as for their number and position (1). Glial cells (or precursors) are present before axonogenesis has started, when some neurons are still differentiating.

To determine whether peripheral glial cells originate, as in vertebrates, from central structures, or from peripheral cells, I have performed a clonal analysis using the *flp* recombinase system. The results show unambiguously that wing glial cells originate from peripheral epithelial cells (2).

The clonal data also show that peripheral glial cells are able to migrate for relatively short distances along the nerve. Migration, which is variable and may encompass several epithelial cells, follows the direction taken by the axons, and starts when axonogenesis is actively taking place. The direction of glial cell migration does not depend on the presence of a general gradient in the epithelium, but on the direction of the underlying axon, suggesting that glial cells use the nerve bundle as substrate for migration. Since migration of glial cells is a feature common to vertebrates and invertebrates, the wing glial cells of *Drosophila melanogaster* represent an ideal genetic model system to investigate the molecular and cellular bases of glial migration. Analysis of gliogenesis in mutants altering a serine-threonine kinase or the Notch molecule, a transmembrane protein carrying multiple EGF repeats, suggests that axonogenesis and glial movements are tightly linked processes requiring cell-cell communication.

Finally, gliogenesis is affected by the same genes involved in the development of sensory organs. Loss of function mutations in proneural genes such as *achaete* and *scute*, two transcription factors of the HLH protein family, induce loss of sensory organs and glial cells. Since the effects are observed at very early stages of glial differentiation, it is likely that these genes act on gliogenesis in the same way they act on sensory organs, i.e., by inducing the differentiation of the precursor cell.

(1) Giangrande et al., (1993) Development 117, 895-904.
(2) Giangrande (1994). Development. In Press.

LOCAL SYNTHESIS OF AXONAL PROTEINS: THE ROLE OF GLIAL CELLS IN THE SQUID GIANT AXON

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The isolated squid giant axon is capable of synthesizing most axonal proteins (Giuditta et al., PNAS 59:1284-1287, 1968), including cytoskeletal proteins (Kaplan et al., Mol. Cell. Neurosci. 3:133-144, 1992) and kinesin. According to one hypothesis (Lasek et al., J. Cell Biol. 74:501-523, 1977), newly synthesized proteins derive from periaxonal glial cells, as the axon is assumed to lack the cytoplasmic system of protein synthesis. However, all the components of this system, including a large family of mRNAs (Giuditta et al., Neurochem. Int. 8:435-442, 1986), and active polysomes (Giuditta et al., J. Neurosci. Res. 28:18-28, 1991) have been detected in the axoplasm of the squid giant axon using biochemical and morphological methods. In a separate set of experiments, newly synthesized axoplasmic RNAs and RNP particles have been identified in the axoplasm or in the perfusate of isolated or perfused giant axons (Rapallino et al., Neurochem. Res. 13:625-631, 1988). On the whole, the data suggest that periaxonal glial cells provide the axon with the RNA components required for the synthesis of axonal proteins.

Functional characterization of tenascin domains with recombinant proteins

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Tenascin (TN) is an extracellular matrix glycoprotein which is transiently expressed by astrocytes in the developing CNS and involved in neuron-glia interactions. TN exhibits a modular structure with aminoterminal EGF type repeats followed by fibronectin type III (FNIII) repeats and homologies to fibrinogen (Fbg) β and γ at the carboxyterminus. Isoforms are distinguished by alternatively spliced FNIII motives between FNIII repeats 5 and 6 and TN monomers are linked to hexamers (hexabrachia) at their aminoterminus. Functionally, TN promotes neuron-astrocyte adhesion, neuron migration and neurite extension. On the other hand, TN embodies repulsive properties for neurons and growth cones. Antibody perturbation studies suggest that these functions are encoded by distinct TN domains. In order to characterize these further, bacterially expressed fusion proteins have been used to map adhesive and counteradhesive domains for neural cells. Fusion proteins based on natural restriction sites showed enhanced degradation and low solubility. Therefore, recombinant proteins matching modular boundaries have been generated from TN cDNA clones using up- and downstream primers designed for PCR and directional cloning into pGEX and pTrcHis expression vectors. FNIII repeats 1-3, 4-5, 6, and 7-8 are soluble in PBS while EGF-type repeats, FNIII A_1-A_2/A_4, BD and the Fbg fragment require 8M urea- containing buffers and subsequent renaturation for purification. Preliminary observations indicate that FNIII 1-3 is adhesive whereas the EGF motive is repulsive for CNS neurons. Primary cells and cell lines of glial origin also adhere to FNIII 1-3. Detailed analysis of recombinant TN fragments will be devoted to cell-interaction sites, complementary interactive ligands and downstream signalling pathways. (Supported by DFG, SFB 317/A2 and a Schilling professorship to AF).

RECRUITMENT OF GLIAL POTASSIUM CHANNELS BY NEURONAL ACTIVITY IN VISCERAL GANGLIA OF HELIX POMATIA.

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One of the essential functions of glial cells is to clear K^+ ions accumulated during neuronal activity. We have developed a method that allows patch clamp experiments to be performed on glial cells kept in place around neurones within molluscan visceral ganglia. The neuronal activity was controlled under current or voltage clamp conditions. The electrical properties of glial membranes were simultaneously recorded in cell-attached or whole-cell patch configurations. Glial cells displayed a more negative resting potential than neurones (-70 vs -53 mV), close to E_K. Neuronal firing induced glial membrane depolarization, directly related to the size of the neuronal outward K^+ current. Glial cells recovered their resting level within one second. Single channel recordings showed that glial membranes contained several types of potassium channels. The predominant channel, present in high density, exhibited an inwardly rectifying behaviour and was moderately sensitive to potential changes. Neuronal firing or voltage clamp depolarizations induced 15-40 s later an increase in both the number and opening probability of active channels. The delay in these events suggested the presence of an intracellular messenger triggered by the neuronal activity. This messenger could be cAMP. Independently of the neurone-induced glial depolarization, the channel recruitment was mimicked by chemicals that raised the intracellular cAMP level (8-bromo-cAMP, IBMX, theophylline, forskolin) as well as by activation of purinergic receptors (ATP, adenosine) and receptors to dopamine, noradrenaline and serotonin. Inwardly rectifying K^+ channels are liable to ensure the entry and exit of potassium ions and thus may play a role in the potassium homeostasis in perineuronal spaces. Our results show that these processes are under the control of a signal released by active neurones.

EXPRESSION OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II MOLECULES ON RESIDENT MICROGLIAL CELLS OF THE ADULT RAT CENTRAL NERVOUS SYSTEM IS STRAIN DEPENDENT.

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Major Histocompatibility Complex (MHC) Class II expression on cultured endothelial cells and astrocytes reportedly correlates with susceptibility to experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis (MS). Resident ramified microglia ($CD45^{low}$ $CD11b/c^+$) were isolated from the normal and inflamed (EAE) adult rat central nervous system (CNS) and analysed for the expression of surface MHC class II molecules by flow cytometry. Isolated microglia from normal LEW CNS were MHC class II negative and the few MHC class II-expressing cells present in the CNS appeared to be $CD45^{high}$ blood-derived cells and not resident microglia. Following EAE induction, the microglia exhibited expression of MHC class II. Parodoxially, we found that a proportion of the microglia in normal adult (EAE-resistant) Brown Norway (BN) rats constitutively express MHC class II Ag (Sedgwick JD et al., J Exp Med 177: 1145, 1993). This contrasts with in vitro data on other CNS cell types and suggests that constitutive MHC class II expression may play a downregulating role, at least on microglia.

STUDIES ON THE PROLIFERATION OF O-2A PRECURSOR CELLS IN RESPONSE TO NEURONES

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During the development of oligodendrocytes from their precursor cells there is a wave of proliferation prior to myelination. Cell culture studies have shown that O-2A precursor cells proliferate in response to co-culture with neurones. It is well established that the precursor cells proliferate in response to a range of growth factors but the nature of the neuronal mitogen(s) is still open. An established murine O-2A precursor line which was generated by immortalising primary O-2A cells with the t-neu oncogene was cultured with cerebellar granule cells. Proliferation was determined by the incorporation of ^{3}H-thymidine. This assay can be carried out in 96-well microtitre plates and can be used for rapid screening of substances (e.g. antibodies) which may interfere with neurone-precursor cell interactions. The cell line exhibited an increased proliferation of up to 90% over basal levels after two days of co-culture with neurones. Similarly, primary O-2A cells showed an increased proliferation above control levels in response to culture with granule cells. We tested the effect of the known O-2A mitogens PDGF and bFGF on the proliferation of the cell line. Neither of these factors alone or in combination or the conditioned medium from the granule cells showed levels of ^{3}H-thymidine incorporation which were comparable to the co-culture results. In addition, separation of the line cells from the granule neurones by a porous membrane allowing the diffusion of soluble factors but preventing direct cell-cell contact resulted in no increased proliferation over the control in the absence of neurones. These results suggest that neuronal cell surface molecules play a major role in the proliferative response of O-2A precursor cells to neurons.

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LONG LASTING ACTIVATION OF GFAP EXPRESSION IN GL15 GLIOBLASTOMA CELL LINE

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The CNS responds to neural injuries with an increase in the number and size of cells expressing GFA protein, i.e. reactive astrocytosis. The induction of GFAP expression is considered the main indicator of astroglial activation. Which is accompanied by hyperplasia and extensive hypertrophy of cell bodies. The specific factors that trigger astrocytes to become reactive and the exact role they play in the injured CNS, are largely unknown. In the present study we evidence by immunohistochemical, Western and Northern analyses, the induction of GFAP expression in GL15 glioma cell line, previously established by us, without addition of any exogenous drug, only leaving the cells for many days in close contact before subcultivation. Following the release of GL15 cells from this prolonged contact, many of them resulted multinucleated. Cell bodies were hypertrophic and numerous thin processes, strongly stained by GFAP extended from them. The activation of GL15 cells so obtained was reproducible, and lasted for more then ten continuous cell passages thereafter, being evocated by a long lasting, not short living signal. This is not generally a glioma cell line property, i.e. in another glioma cell line, GL22, previously established by us, GFAP expression and morphological changes were not inducible under the same culture conditions. The in vivo relevance of our data must still be assessed. Is the different in vitro reply of GL15 and GL22 cells to the same induction process, representative of two different types of astrocytes in vivo. Or is the different behaviour of GL15 and GL22 cells due to different genetic mutations, epiphenomena of the malignant transformation. Nonetheless GL15 cells represent a simple sistem of astrocytic response to a modification in the culture environment in the absence of interactions with neurons and a tool for investigating in more detail the mechanisms involved in triggering glial reactivity.

BIRD-SONG FOR "GLIOLOGISTS"

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Bird song is a well established tool for studying brain-behavioral relationship. These previous studies were mainly focused on morphological and functional aspects of neurons. Here, we report that seasonal changes in song structures are paralleled by alterations in glial differentiation and activity.

Adult male canaries were analysed by classical histology and immunohistochemistry against glial cells in the reproductive period and during the autumnal quiescence. Song quality has been assessed by sonagraphic analyses. Our data give hints that the glial cell populations of the vocal control system change seasonally which may influence the well documented plasticity of neurons[1)] in this functional system. Thus, glial cells may be involved in controlling the motor outflow (song).

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GLIAL CELL VOLUME AND PH_i IN LACTACIDOSIS

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Introduction: In cerebral ischemia, the tissue pH may fall to 6.0 or below, with lactic acid concentrations reaching 20-30 mmol/kg. Acidosis is a potent factor of cytotoxic swelling and damage of nerve and glial cells. Since the acid/base homeostasis of these cells is likely to be affected, the significance of lactacidosis was currently examined in vitro with regard to changes of the cell volume and of the intracellular pH (pH_i) of glial cells.

Methods: C6 glioma cells and astrocytes were harvested from culture und suspended under control of pH, temperature, and pO_2. Cell volume and pH_i were assessed by flow cytometry using BCECF as pH-indicator. Following a control period, the suspension was titrated from pH 7.4 to 7.0, 6.8, or 6.2 by isotonic lactic acid. Cell volume and pH_i were measured during acidosis (60 min) and after restoration of the medium pH to normal (30 min). Further, acidosis experiments were performed with administration of amiloride, or employment of Na^+-free medium.

Results: Acidification to pH 6.8 or below led to immediate swelling of C6 glioma cells and of astrocytes. Cell swelling amounted to 106-107% at pH 6.8, while to 120% at pH 6.2 ($p<0.01$). Neither C6 glioma cells nor astrocytes were capable of defending their normal intracellular pH. pH_i of C6 glioma cells fell to 6.5 at an acidosis level in the medium of pH 6.2. Intracellular acidosis was more pronounced in the primary cultured astrocytes. Following normalization of the medium pH to 7.4, pH_i returned to the control level within few minutes, whereas recovery of the cell volume was incomplete. Suspension of glial cells at pH 6.2 with amiloride or in a Na^+-free medium led to reduction or complete inhibition of cell swelling, while the intracellular acidosis was more severe.

Discussion: The present findings confirm the significance of lactacidosis in cytotoxic swelling of glial cells. The cells were unable to maintain a normal pH_i at levels of extracellular acidosis, which occur under pathophysiological conditions in the brain. Inhibition of glial swelling from acidosis by Na^+-free medium indicates a major role of Na^+/H^+-exchange mechanisms in the swelling process, whereas the antiporter seems to have a limited function for maintenance of a normal pH_i in acidosis.

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DIGOXIGENYLATED PRIMARY ANTIBODIES AND THE EPOS-TECHNIQUE FOR MULTIPLE LABELLING OF GLIA, VESSELS AND β-AMYLOID IN THE HUMAN BRAIN

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Several neurodegenerative diseases are associated with multiple pathological changes of astroglia, microglia and blood vessels. The complexity of these alterations e.g. in Alzheimer's disease (AD) requires an adequate cytochemical methodology adapted to the autoptic human brain. Therefore, an essential purpose of this study was the elaboration of versatile protocols for double and triple labelling of glial and vascular components in normal and pathologically altered brain tissue. These methods are based on the immunocytochemical detection of glial fibrillary acidic protein (GFAP) in astrocytes, leukocyte common antigen (LCA) in microglia, CD-34 in vessels and β-amyloid in senile plaques of AD. To facilitate the simultaneous visualization of these specific markers novel, non-intricate techniques are introduced in addition to conventional methods like ABC-, PAP- and APAAP-techniques.
In the present study we applied for the first time the digoxigenin-anti-digoxigenin-(DIG-)technology with its advantageous properties to immunocytochemistry. Astrocytes were visualized using an approach based on digoxigenylated rabbit-anti-GFAP followed by sheep-anti-digoxigenin conjugated to peroxidase and diaminobenzidine (DAB) as chromogen. This labelling of astroglia was combined with the detection of either β-amyloid-immunoreactivity (-ir), LCA-ir or CD-34-ir applying a dual-peroxidase method with nickel-enhanced DAB and plain DAB as chromogens which produced well-contrasting black and brown reaction products, respectively. Furthermore, microglia were demonstrated by the novel Enhanced Polymer One-Step-Staining-(EPOS-)technique using polymers bound to primary antibodies (e.g. to LCA) as well as to the marker enzyme. In triple-label experiments, microglia and astroglia were revealed by a dual-peroxidase procedure which was followed by the pink staining of vessels after applying the APAAP-method and naphthol-AS-MX-phosphate/Fast Red TR as substrates.
The demonstration of spatial relationships between glial and vascular components in normal tissue and their alterations e.g. in AD might provide further insights in time course and localization of pathological events.

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CHRONIC CENTRAL ADMINISTRATION OF INTERLEUKIN-2 CAUSES NEUROTOXICITY AND GLIOSIS

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The immunoregulator interleukin-2 (IL-2) has been introduced in clinical practice to fight peripheral and brain tumors (Rosenberg, Sci Am 262 (5): 62-69,1990). However, those patients often develop severe neurologic, neuropsychiatric and neuroendocrine side effects. In an animal model of chronic intracerebroventricular administration of IL-2 in rats, we showed recently a long lasting activation of the hypothalamic pituitary adrenal function (Hanisch *et al.*, Soc. Neurosci. Abstr. 19:209.9, 1993). Examination of brain tissue sections of IL-2 treated animals (5 units/hr for 14 days) revealed pathological alterations. Macroscopically, brains exhibited a massive enlargement of the ventricle ipsilateral to the infusion site, apparently due to a damage to the surrounding tissue. Immunostaining revealed a decrease in myelin basic protein (MBP) in the vicinity of the injection site and a pronouced increase in the number of glial fibrillary acidic protein (GFAP)-positive astrocytes in the periventricular tissue. Furthermore, quantitative neurotransmitter determination and radioligand binding studies showed changes in certain neurotransmitter systems in both proximal and remote brain areas. The results indicate that chronically increased central levels of IL-2 as associated with immunotherapeutic application of the cytokine can also lead to cytotoxic outcomes paralleled or preceded by functional disturbances. Supported by HFSPO (U.-K. Hanisch).

ASTROGLIAL 5-HT$_2$ RECEPTORS, VISUALIZED WITH ANTI-IDIOTYPIC ANTIBODIES, ARE LINKED TO PHOSPHOLIPASE C AND A$_2$ DEPENDENT RISES IN [Ca^{2+}]$_i$.

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Potential involvement of voltage-operated calcium channels (VOC:s) in 5-hydroxytryptamine (5-HT)-induced rises in intracellular calcium levels ([Ca^{2+}]$_i$) in type 1 astrocytes in primary cultures was investigated using the calcium sensitive probe fura-2. Also the possible contribution of phospholipase C (PLC) and A$_2$ (PLA$_2$) to the 5-HT-evoked calcium responses was examined. Furthermore, distribution of 5-HT$_2$ receptors on the cell membrane of the astrocytes was investigated using anti-idiotypic antibodies. The L-, N-, T- and P- VOC antagonists nifedipine, ω-conotoxin GVIA, ethosuximide and funnel web spider toxin (FTX) were used after depolarization with high extracellular potassium levels (56 mM) for the purpose of characterizing possible VOC:s in astrocytes. L-, N- and T-channels were found to be expressed in the astrocytes. However, no P-channels were found.

The results suggest that the mechanisms behind 5-HT-induced elevation of [Ca^{2+}]$_i$ are not involving VOC:s. Instead it appears that PLC and PLA$_2$ constitute, direct or indirect, important regulators of both the transmembrane flow and release of calcium from internal stores. Furthermore, the results also indicate a potential coupling of 5-HT$_2$ receptors to PLC and also to PLA$_2$ via a pertussis-insensitive G-protein in the astrocytes.

METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION INDUCES ASTROGLIAL SWELLING

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Activation of metabotropic glutamate receptors (mGluRs), coupled to the phosphoinositide (PI) or adenylate cyclase/cyclic AMP systems, induced a rapid cell volume increase in type 1 astroglial cells in primary culture from cerebral cortex of newborn rat. Rapid relative volume changes (within seconds) in single cells were examined by microspectrofluorimetry after loading the cells with the highly fluorescent intracellular probe fura-2/AM. At its isosbestic point, 358nm, fura-2 is ion-insensitive and the fluorescent signals emitted related only to the intracellular dye concentration.

The present data suggest that activation of mGluRs can induce swelling of single type I astroglial cells. These receptors are coupled to different signal transduction routes; one including IP$_3$ synthesis, intracellular Ca^{2+} increase and opening of a delayed outward K$^+$ rectifier, and another activating a G$_i$-protein and opening an inward K$^+$ rectifier. Both molecular pathways then affect at least one Na$^+$-K$^+$-2Cl$^-$-cotransporter and a Na$^+$-K$^+$-ATPase, which then might affect the electrogenic Na$^+$-dependent Glu carrier, through which Glu is taken up into the astrocytes. The Glu induced astroglial swelling is not only the result of these two processes, but requires another, until now unidentified mechanism, probably some ketamine sensitive K$^+$-outflux or Na$^+$-influx.

MODIFICATIONS OF GLIAL METABOLISM OF GLUTAMATE AFTER SEROTONERGIC NEURONS DEGENERATION IN THE HIPPOCAMPUS OF THE RAT. H. HARDIN *, A. BERNARD, F. RAJAS °, P. CORONAS, E. DERRINGTON, M.F. BELIN and M. DIDIER-BAZES.INSERM CJF 90-10 and ° INSERM U369, Faculté de Médecine A. Carrel, rue G. Paradin, 69 372 LYON Cedex 08, FRANCE. Fax : (33) 78. 77. 86. 12.

Interactions between neurons and astrocytes participate to the central nervous system homeostasis. In particular, the excitability of the hippocampus, which principally depends on GABA and glutamate levels, may be controlled by astrocytes since they catabolize glutamate (released by neurons or produced by the degradation of GABA) into neutral metabolites by glutamine synthetase (GS) and glutamate dehydrogenase (GDH) pathways. Astrocytes could be modulated by several signalling molecules originating from neuronal inputs. It has been proposed that the degeneration of serotonergic neurons could perturbate the functional interactions between neurons and astrocytes and impair hippocampal glutamatergic and GABAergic transmission. In order to investigate the role of serotonergic neurons on the astrocytic catabolism of glutamate, we have analyzed GS and GDH expression in the hippocampus after the degeneration of serotonergic neurons by a specific neurotoxin (5.7 DHT). This treatment caused a reactive gliosis with hypertrophy (increase in GFAP expression) but not hyperplasia of astrocytes. The expression of GDH was specifically and highly induced in the hippocampus whereas the steady state of GS remained unchanged. Glutamate metabolism appears preferentially regulated by a control of GDH expression rather than GS. Serotonin is not the main factor involved in this control of GDH expression since the inhibition of serotonin synthesis (by pCPA administration) induced only a slight increase of GDH level. The role of other signalling molecules related to serotonergic neurons degeneration is discussed.

QUANTIFICATION OF GLIOSIS IN SCHIZOPHRENIA

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Various structural anomalies as revealed by morphometric methods in the schizophrenic brains have been reported. However, attempts to assess the presence of gliosis in schizophrenia have led to controversy. It is well known that the production of glial fibrillary protein (GFAP) increases prominently along with the astrocytic response triggered by neuronal damage. This study was undertaken to investigate the extent of astrogliosis through quantifying immunoreactivity of GFAP in 5 schizophrenic post mortem brains compared to that of 4 nonpsychotic controls. 7 μm thick sections taken from thalamus, nucleus ruber, gyrus temporalis inferior, nucleus caudatus, amygdala, substansia nigra, gyrus precentralis, gyrus temporalis superior, pallidus and cerebellar cortex were stained with GFAP Histomune Immunostaining system (Monoclonal antibody (Murine) 595074 and 580000, Cambridge Research Laboratory). Micrographs and Data were evaluated by NIH Image V1.33g and Statgraph, respectively. Schizophrenic amgdala, frontal pole and superior temporal gyrus expressed significantly ($p<0.05$) increased GFAP densities whereas no alterations were detected in the other regions studied. We consider our findings relevant to the course of impairment in schizophrenia on the basis that areas like prefrontal cortex and amygdala are primarily involved in the regulation of behavior and memory which are affected in schizophrenia.

PEPTIDE N-GLYCOSIDASE F MODIFIES IMMUNODETECTION OF A2B5 REACTING COMPONENTS IN 5 DAYS OLD C57/Bl 6 MICE BRAIN LIPID EXTRACT.

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The A2B5 antibody has been reported to react with several ganglioside antigens in neuronal tissues and on HPTLC plates, and with the highly sialylated form of N-CAM. It is present on neurons and on the surface of progenitors of oligodendrocytes. Brain lipid extract was chromatographed on silica gel HPTLC plates from mice aged 5 days, 18 days and adult, using chloroform-methanol-CaCl2 O.25% 50:40:10 (V/V/V/). A2B5 immunodetection was performed on HPTLC using peroxidase-labelled second antibody and 1-chloronaphtol reagent. At every age studied, several glycosylated components reacting with A2B5 were found. . N-glycosidase F, recombinant, was used on the lipid extract. After 18 hours hydrolysis at 37°C in buffer, salts were eliminated using a Sephadex G25 column; no loss of gangliosides was found, at different steps, using Fidia ganglioside mixture as control. Lipid extracts were rechromatographed after hydrolysis and A2B5 immunodetection was performed as previously. In the lipid extract, although some TLC bands reacting with A2B5 remained, several A2B5 reacting antigens had disappeared, especially in the area corresponding to di- and trisialylated gangliosides. Modifications were especially marked in brain lipid extract of 5-day old mice. Relation of these N-glycosidase labile components with oligodendrocyte lineage differenciation are under investigation.

Ecto - ß(1,4)Galactosyltransferase is a Mediator of Neuron-Glia Cell Adhesion

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The directed migration of neurons along cellular guides as well as their highly selective grouping into distinct laminae or nuclei have initiated a still ongoing research in molecular adhesion mechanisms during brain development. Thereby, we have focussed on the possible role of an ectoenzyme, the galactosyltransferase EC 2.4.1.38, that has been hypothetically linked to the Reeler malformation of cortical development in mice. Using a polyclonal antiserum raised in our laboratory, we have been able to localize this enzyme on cell membranes of the ventricular zone and developing cortical plate as well as the external granular layer and the subjacent migratory pathway of the cerebellar cortical anlage. In cerebellar primary cultures, the antibody stains cell mebranes of migrating neurons, whereas glial cells are immunonegative. Specific interference with enzyme activity in vitro influences cell migration and eventually leads to cell detachment from the substratum. These data provide evidence for a key role of this ectoenzyme in brain development. Moreover, genetic defects of genes regulating enzyme expression and/or activity have been described, that could account for genetic defects selectively interfering with brain development like the Reeler mutation in mice.

EXPRESSION OF MYELIN/OLIGODENDROCYTE GLYCOPROTEIN (MOG) IN RAT BRAIN GLIA CULTURES

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The MOG-specific monoclonal antibody (mAb) 8-18C5 amplifies demyelination and modifies the clinical expression of EAE in rats and mice. MOG is detectable in vivo at day 15 postnatally (p.n.) with a lag of 2 days after Myelin-Basic-Protein (MBP). MOG-mRNA can be detected from day 11 p.n. and has its highest expression at day 15-25 p.n.
We have investigated the expression of MOG in glia cell cultures derived from cerebral hemispheres of newborn rat brains. Oligodendrocytes in these cultures are characterized by the coordinated expression of myelin constituents, e.g. MBP, PLP, Wolfgram-Protein, Galactocerebroside and myelin associated glycoproteins MAG and MOG. Indirect immunfluorescence studies with mAb 8-18C5 indicate that oligodendrocytes were positively labeled after 1 week in vitro and MOG was expressed on the cell body. Lateron (> 2 weeks) it is also detectable on the large cellular extensions. Western blot analysis indicated that in 1 week old cultures a 26/28 kDa component was the predominant constituent. With prolonged time of culture, a shift from the 26/28 kDa component to a 53 kDa protein was observed. The 53 kDa band was induced after 2 weeks in vitro. Concomitantly, the 26/28 kDa band disappeared. This shift was independent on the postnatal age of the cells but correlated with the time in vitro, and might possibly signify degenerative alterations in cultured oligodendrocytes.
This work was supported by the Tönjes-Vagt-Stiftung.

RECIPROCAL MODULATION OF GANGLIOSIDES AND SOLUBLE GROWTH FACTORS IN CULTURED RETINAL MÛLLER GLIAL CELLS

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As glycoconjugates are involved in numerous aspects of cell behaviour, cultured retinal Müller glial (RMG) cells were characterized according to their composition by gangliosides (Gg) (sialylated glycosphingolipids). RMG cells exhibit a Gg composition dominated by simple monosialoGg types (40% GM3, 23% GM2, 10% GM1), with the balance mainly GD1a (15%). This profile resembles other in vitro glial types (eg. astrocytes) but differs from the situation observed in cultured neurons, which possess significant amounts of complex Gg. Treatment of RMG cell cultures pre-loaded with ^{3}H-galactose or ^{3}H-glucosamine with the neurotrophic factor basic fibroblast growth factor (bFGF) (10ng/ml) leads to a 4-fold increase of precursor incorporation into Gg, the majority of the increase occurring in GM3. Similarly, bFGF increases the absolute level of Gg per unit protein (about 50% control levels, n=4). Epidermal growth factor (EGF) has similar, albeit smaller, effects. Hence these data indicate for the first time an inductive effect of certain growth factors on Gg synthesis, which would have important consequences for cellular metabolism. Moreover, EGF and bFGF are both potent mitogens for RMG cells in vitro, and we demonstrate here that exogenous Gg modify the response of these cells to exogenous growth factors, and that these modifications depend both on the specific factor and the specific Gg in question. In general, mixed brain Gg and GM1 are inhibitory, GM3 is without effect and GD3, GD1a and GT1b are stimulatory. This modulation seems to be mediated at the level of the respective receptors, as witnessed by parallel modifications in phosphotyrosine activation. Thus we speculate that these two systems of signalling molecules may be mutually linked at different molecular levels.

COLOCALIZATION OF NEUROTRANSMITTER RECEPTORS ON ASTROCYTES OF VARIOUS REGIONS OF RAT CNS

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There is strong evidence that astrocytes in the mammalian CNS express receptors for a variety of neurotransmitters (1). We have been interested to investigate whether receptors for various neurotransmitter systems are colocalized on the glial membrane. By means of electrophysiological, immunohistochemical and autoradiographic techniques we have studied the expression of cholinergic, adrenergic and peptidergic receptors on astrocytes in explant cultures of various areas of rat CNS. Combined immunohistochemical and autoradiographic studies have demonstrated that a great number of astrocytes were immunostained by the monoclonal muscarinic antibody M35 and simultaneously labelled either by ^{3}H-noradrenaline, the β-adrenergic antagonist ^{3}H-dihydroalprenolol, the peptides ^{125}I-angiotensin, ^{125}I-neuropeptide Y, ^{3}H-cholecystokinin or ^{125}I-bombesin. Electrophysiological studies indicate that on the same astrocyte the cholinergic agonist muscarine and the β-adrenergic agonist isoprenaline as well as the gastro-intestinal peptides cholecystokinin and bombesin caused a hyperpolarization whereas noradrenaline and the vasoactive peptides angiotensin II, bradykinin and neuropeptide Y produced a depolarization. From these results it is suggested that there is a coexistence of cholinergic, adrenergic and peptidergic receptors on the glial membrane.

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SYNTHESIS OF CARNOSINE BY OLIGODENDROCYTE- AND ASTROCYTE-ENRICHED PRIMARY CULTURES

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Carnosine (ß-alanyl-histidine) and structurally related dipeptides such as anserine (ß-alanyl-histidine) and homocarnosine (γ-aminobutyryl-histidine) are well known constituents of brain and muscle. In order to study the biosynthesis of carnosine by glial cells (1) in more detail, oligodendrocyte- and astrocyte-enriched cultures were prepared as described by McCarthy and de Vellis (2). After cell separation and plating on poly-L-lysine coated dishes, the oligodendrocyte-enriched cultures were maintained for 3 days in serum free medium (3) containing insulin (5 μg/ml), transferrin (5 μg/ml) and selenite (30 nM) as additives. The astrocyte-enriched cultures were kept in DMEM-medium supplemented with 10% FCS. Immunofluorescence-staining against GalC and GFAP revealed that both cultures contained about 95% of the respective cell type.

To study the biosynthesis of carnosine, radiolabeled ß-alanine (1 μCi/ml) was added to the culture medium. After incubation for 6 hours the cells were washed with PBS and then lysed by addition of methanolic acetic acid. The cell extracts were fractionated by ion-exchange chromatography to separate ß-alanine and carnosine (1). Counting for radioactivity revealed that about 20% of the radioactive precursor was taken up by both cell cultures. While only trace amounts of carnosine were isolated from the extracts of astrocyte-enriched cultures, more than 30% of the total radioactivity contained in the cell extracts of the oligodendrocyte cultures could be isolated as radiolabeled carnosine. These results indicate that carnosine is preferentially, if not exclusively, synthesized by oligodendrocytes.

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EFFECT OF αMSH AND THE ACTH$_{4-9}$ ANALOGUE ORG 2766 ON THE REGULATION OF THE LOW AFFINITY NGF-RECEPTOR ON SCHWANN CELLS

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The neurotrophic peptides αMSH and Org 2766 accelerate functional recovery of the sciatic nerve after a nerve crush. Such damage leads to down regulation of the nerve growth factor receptor on sensory axons, proliferation of Schwann cells and an increased expression of the low affinity NGF-receptor (LNGFR) in these cells. Enhancement of nerve regeneration by neurotrophic peptides might be due to an effect on Schwann cells, which play an important role in the regenerative response. We investigated the effect of αMSH and Org 2766 on Schwann cell division (BrdU incorporation) and LNGFR expression *in vitro*. Cells were also treated with forskolin, as it is known that forskolin stimulates peripheral regeneration and regulates the expression of LNGFR expression on glial cells. Schwann cells were isolated from rats (p5) and a cell line was established. Characterisation of the cells showed that >99% of the cells were S-100 and LNGFR positive. None of the cells were thy1.1 positive (a marker for fibroblasts). Cells were cultured in 96-wells plates and treated with αMSH, Org 2766 (0.1-100 nM) or forskolin 10 μM. After 24 h BrdU was added (final concentration of 10 μM), the cells were fixed after 17h and processed for BrdU and LNGFR ELISA. Forskolin increased the BrdU incorporation with 28±9% and the LNGFR production with 289±10%. αMSH and Org 2766 had no effect on BrdU incorporation and LNGFR expression. This may be due to a differentiated (expressing myelination-related proteins) state of the Schwann cells, as all cells were myelin basic protein positive. Thus, the neurotrophic effect of αMSH and Org 2766 is probably not mediated through a change in cell division and LNGFR expression in the Schwann cells.

A NOVEL MYELIN GENE IS SPECIFICALLY EXPRESSED IN OLIGODENDROCYTES

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The elaboration of the myelin sheath is an essential process for the correct function of the nervous system in vertebrates. Several genes and their proteins have been described that are involved in this task. Loss of their function leads to extensive disorders in the brain. In the central nervous system (CNS) the myelin is produced by oligodendrocytes. Here we report the characterization of a novel gene which is specifically transcribed in this glial cell type and generates two major mRNAs of 2.6 and 4.1 kb. We have identified rat cDNA clones tentatively named CNS1 and COP-25.1 corresponding to these transcripts. In their 5' region the two clones share a 420 bp region of sequence identity which suggests that they are generated by alternative splicing. A putative open reading frame is located within this area that codes for a 10 kDa CNS1 protein and reads in the yet uncharacterized 5' end of COP-25.1. Northern hybridization revealed that both mRNAs are expressed initially in parallel in the postnatal brain stem and spinal cord. However, COP-25.1 expression peaks with the event of myelination at postnatal days 15-21 and decreases afterwards, whereas CNS1 remains at high levels in the adult. *In situ* hybridization performed on brain, optic nerve and spinal cord sections demonstrated exclusive expression in oligodendrocytes. Moreover, during development CNS1 and COP-25.1 expression follows the myelination process and the gene expression of myelin basic protein (MBP) and proteolipid protein (PLP). However, expression of the novel myelin gene is delayed as compared to the structural myelin genes MBP and PLP. Interestingly, CNS1 and COP-25.1 mRNAs appear to be differently sublocalized within the oligodendrocyte: COP-25.1 message is distributed primarily in the perikaryon, whereas CNS1 mRNA is transported into the processes of the cell.

CHARACTERIZATION OF A BRAIN DEVELOPMENTAL PROTEIN EXPRESSED BY SUBVENTRICULAR AND GLIAL CELLS IN ADULT RAT BRAIN. J Honnorat, M Aguera, C Vocanson, MF Belin and JC Antoine. INSERM CJF 90-10, Lab. Anat. Pathol. Fac. Alexis Carrel, 69372 Lyon, France.

Several types of autoantibodies have been reported in association with paraneoplastic neurological syndromes. These autoantibodies give an opportunity to improve the knowledge of these syndromes as well as to characterize specific proteins of the nervous system, unknown until now. We have reported a patient with severe posterior uveitis and paraneoplastic encephalomyelitis whose serum and CSF IgG reacted with a protein (called CV2) common to retina and nervous system. This protein was strongly expressed during embryogenesis throughout the central and peripheral nervous system except for the ventricular zone where progenitor cells, undergoing mitosis, are located. The expression of this protein is reduced by cell maturation. In the adult brain, CV2 expression persisted in some structures only. It was strongly expressed in cells of the immature subventricular layers of the dentate gyrus, olfactory bulb and lateral ventricle and in cells indentified as glial cells of the white matter in the cerebellum and in the cortico-spinal tract. By western blotting, this protein has an apparent molecular weight of 65 and 45 kDa. These results suggest that CV2 protein could play a role in the nervous system maturation and in glial metabolism in adult brain. Furthermore, Purkinje cells degeneration without inflammation in the cerebellum of the patient suggest that this protein had a role in neuronal survival.

Cortical and spinal cord type I astrocytes influence neurite outgrowth of cortical neurons.

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The potential influence of either astrocytes type I derived from P4 cerebral cortex (C-A) or from P4 cervical spinal cord (SP-A) on the neurite outgrowth of P1 cerebral cortex neurons was studied using an *in vitro* coculture system. Both the influence of astrocyte type I conditioned medium and astrocyte type I feederlayer was investigated. Confluent astrocyte type I cultures (C-A or SP-A) were used as feederlayers. The same cultures were used to create ACM. Chemically defined medium (CDM) was added to these cultures for 72 hours.
The quality of astrocyte type I cultures were examined by immunocytochemical identification, using different glial cell type markers : anti-glial fibrillary acidic protein (GFAP, 1:400, DAKO), anti-A2B5 (1:10, MoAb IgM), anti-galactocerebroside (GC, 1:50). In addition the fibroblast markers anti-Thy1.1 (1:250, Pharmingen) was used. In rat optic nerve type I astrocytes are GFAP-positive and type II astrocytes are both GFAP- and A2B5-positive (Raff et al., 1983a,b). Oligodendrocytes are GC positive. Based on our immunocytochemical data we conclude that both astroglia cultures (C-A and SP-A) mainly consist (> 95%) of GFAP-positive type I astrocytes.
To study the effect of the various astroglia populations on cortical neurite formation and outgrowth various neuronal markers were used : MAP-2 as a dendrite marker and NF-H-SMI-31 as an axon marker. For quantification of the data a IBAS-2000 (KONTRON) system was used.
Preliminary observations indicate that dendrite as well as axon outgrowth of P1 cerebral cortical neurons is influenced by cortex and spinal cord astroglia type I cultures.

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REGULATION OF MYELIN GENES EXPRESSION IN THE NEONATAL HYPOTHYROIDISM.

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Lack of thyroid hormone (T3) during brain development causes profound alterations in neuronal populations and oligodendrocytes. T3 action is exerted through interaction with specific nuclear receptors (TR) that act as ligand-dependent transcription factors. There are several isoforms encoded by two different genes α and β.TR are able to form heterodimers with other proteins, the best known is the 9-cis retinoic acid (9-cis-RA) receptor (RXR), that function as coactivator by increasing the hormonal response mediated by TR. Neonatal hypothyroidism reduced the expression of the three major myelin protein genes: Myelin associated glycoprotein (MAG), myelin basic protein (MBP) and proteolipid protein (PLP), both at mRNA and protein level, but unexpectedly we have found that there were spontaneous recovery of their mRNA and protein levels during development. Meanwhile the O2A progenitors express the TRα isoforms,TRβ expression coincides with the oligodendrocyte differentiation, previous to MBP expression, raising the possibility that unliganded TRβ would control the transcriptional activation of the myelin genes. In order to study the mechanism(s) involved in the T3-independent induction of these mRNAs, we have used transient transfection assays with the MBP promoter in C6 glioma cells, which contains a T3 response element.

Our results shown that the effects of either TRα or TRβ were T3-dependent. Furthermore, heterodimers of the any RXR isoforms (α or β) with TRα or TRβ did not stimulate the basal activity of this promoter, therefore unliganded TR homodimers or TR-RXR heterodimers are not responsible for the recovery in the expression of myelin genes. Activation by 9-cis RA and enhance T3-dependent transcriptional activation of MBP promoter was specific for the heterodimer TRβ-RXRα. These results shown that the activation by 9-cis-RA of the RXRα-TRβ heterodimers is one of the mechanisms for the spontaneous recovery of the myelin gene expression and this effect is dependent on the TRβ expression.

V-erbA ONCOGENE INDUCES AUTOCRINE GROWTH STIMULATION OF A GLIAL CELL LINE

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Using recombinant retroviruses, we have introduced and expressed the c-erbA and v-erbA genes coding respectively for the normal and mutated thyroid hormone (T3) receptor in a v-myc-immortalized mouse glial cell line (B3.1) established from embryonal brain. B3.1 cells exhibit a mixed glial phenotype expressing astrocyte and oligodendrocyte markers. They do not grow but survive in serum-free medium (N2) for several days. Upon v-erbA expression, these cells show an extended survival capacity and are able to grow in the presence of IGF or high insulin concentrations. In contrast, the c-erbA proto-oncogene leads to a complete blockade in DNA synthesis and cells rapidly degenerate and die in serum-free medium. This effect is independent of the presence of IGFI or insulin. Normal, v-erbA- and c-erbA-expressing B3.1 cells respond by the mitogenic stimulation by bFGF and PDGF and also but to a lesser extend of TGF alpha. IGFI is however only able to potentiate the action of these two factors but has no mitogenic activity per se. Transfer of conditioned medium from v-erbA-expressing cells to c-erbA cells increase their survival and stimulate DNA synthesis, particularly in the presence of insulin or IGF-I. These results suggest that while c-erbA causes a growth arrest, v-erbA induces the synthesis and secretion of putative survival/growth factor(s) in the glial B3.1 cells leading to an autocrine growth stimulation. Efforts are currently ongoing to characterize this factor(s).

REGULATION OF THE INWARD RECTIFIER IN MICROGLIAL CELLS BY G-PROTEINS AND INTRACELLULAR CALCIUM

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An inwardly rectifying current (K_{IR}) has been found to be the only voltage-activated current in unstimulated microglial cells, both in culture and in brain slices (Kettenmann et al.1989, Kettenmann et al. 1990, Brockhaus et al. 1993). The membrane potential and electrical responsiveness of microglia is, therefore, strongly dependent on the expression and function of this current. In this study the influence of GTPcS, an activator of G-proteins, and changes in the intracellular calcium concentration, on K_{IR} were investigated. Whole-cell voltage-clamp recordings were combined with both intracellular perfusion and calcium-imaging using the calcium sensitive fluorescent dye Fura-2. An interaction of K_{IR} with the receptor mediated response to ATP and ADP (Walz et al. 1993) was also investigated as an example of possible physiological modulation of microglia electrophysiology.

Both GTPcS and a rise in intracellular calcium (to 3 M) were able to independently and completely block K_{IR}. ADP and occasionally ATP were also found to weakly inhibit K_{IR}.

These results show that the K_{IR} in microglia cells can be blocked by a rise in the intracellular calcium concentration or activation of G-proteins. This suggests that physiological stimuli, such as an increase in the extracellular ATP or ADP concentration, could modulate the expression of the K_{IR} in these cells.

ATP INDUCES AN INCREASE OF INTRACELLULAR CALCIUM IN CULTURED MOUSE MICROGLIAL CELLS

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Microglial cells are not considered as elements of a fast signal transfer in the nervous system and thus as cells expressing neurotransmitter receptors. In this study, however, we found that the cotransmitter ATP (10^{-7}M to 10^{-4}M) induced a transient, rapid rise in intracellular [Ca^{++}] in cultured microglial cells from mouse cortex. 30s application of ATP induced a rise in intracellular calcium with a peak after 20s as measured with a fura-2 based Ca^{++} imaging system. Subsequently resting Ca^{++} levels were restored within several minutes. ATP was more potent than ADP, while AMP and adenosine were uneffective indicating the involvement of P_2 receptors. The lack of an ATP-induced Ca^{++} response in Ca^{++}-free bathing solution points to an Ca^{++} influx; this influx is not mediated by voltage-gated Ca^{++} channels, since depolarization (by 50mM K^+) did not trigger a Ca^{++} elevation. Simultaneous current recordings with the perforated patch clamp technique indicates that the rise in Ca^{++} is accompanied by an inward current. The ATP-induced response could be a means for microglia to sense neuronal activity. Moreover, it could serve as a mediator for detecting injured neurons which may leak ATP; the rise in Ca^{++} could induce a cellular response in microglial cells, e.g. such as secretion, migration or phagocytosis.

SEAD CELLS IMPROVE FUNCTIONAL RECOVERY AND HOST SPROUTING RESPONSE FOLLOWING SELECTIVE 6-OHDA LESION IN THE RAT. E.L. Imperato*, K. Sakai, G.M. Seigel, and J.T. Hansen. Department of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642, * Institut für Hirnforschung, Universität Zürich, August-Forelstr. 1, CH-8029 Zürich

The underlying neuropathology of Parkinson's disease is characterized by a decrease in substantia nigra pars compacta dopamine (DA) neurons that results in a depletion of DA innervation in the striatum. One approach to ameliorating the loss of DA innervation is to use a graft as a source of neurotrophic factors to induce a host sprouting response from spared DA neurons. In order to further examine the characteristics involved in enhancing graft survival or host recovery, we examined a Schwann cell line, SEAD cells, *in vitro*. SEAD cells were developed by infecting primary rat Schwann cells using a retroviral vector containing the 12S E1A gene. SEAD cells express the characteristic Schwann cell markers S-100, nerve growth factor receptor (NGF-rec), and insulin-like growth factor-1 (IGF-1) as detected immunocytochemically. Tumorigenicity tests *in vivo* and *in vitro* suggest no contact inhibition of growth (in nude mice) yet an adherence dependence for growth of the SEAD cells (in soft agar). We evaluated the efficacy of grafted SEAD cells to enhance a host sprouting response and functional recovery in a selective unilateral 6-hydroxydopamine (6-OHDA) lesioned rat model of Parkinson's disease. No tumors were observed in any of the grafted animals. SEAD cell graft recipients showed significant improvement in amphetamine-induced rotational behavior after selective lesioning ($\alpha<0.02$) and an increase in tyrosine hydroxylase immunoreactive fibers ventromedial to the graft. Although several animals showed SEAD cell survival, overall graft survival was poor and did not correlate with functional recovery. Possible explanations for the observed host sprouting response and functional recovery may be graft derived neurotrophic factors or host derived factors related to the truama of transplantation.

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IMMUNOGLOBULIN G UPTAKE AND THE ASTROCYTE REACTIVE PROLIFERATION IN THE BRAIN OF ADULT MICE

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Cell divisions were labeled with [^{3}H]thymidine injected at different intervals following the injury. Brain sections were doubly immunostained for GFAP and for immunoglobulin G, and subjected to autoradiography. Thereafter, locations of two types of autoradiographically labeled astrocytes were recorded: (1) immunopositive both for GFAP and IgG (GFAP+/IgG+) and (2) immunopositive exclusively for GFAP (GFAP+/IgG-). Locations of autoradiographically unlabeled GFAP+/IgG+ astrocytes were also recorded.

Reactive proliferation of GFAP+/IgG- astrocytes began on the 1st day after injury and reached its maximal intensity on day 4 after injury, whereas the population of non-proliferating GFAP+/IgG+ astrocytes increased continuously during 8 days after injury then decreased. However, during the whole examined period only occasional proliferating GFAP+/IgG+ astrocytes were found.

These results allow to conclude that the ability of astrocytes to proliferate after IgG uptake was very low.

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DIFFERENT PATTERNS OF PROLIFERATIVE ACTIVITY OF GFAP AND S-100 PROTEIN-IMMUNOPOSITIVE ASTROCYTES IN THE RAT BRAIN INJURED DURING THE EARLY POSTNATAL PERIOD.

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The brain was injured on the 6th day of postnatal development. The animals were injected with [^{3}H]-thymidine at different intervals following the injury and killed 40 days later. Brain sections were immunostained for glial fibrillary acidic protein (GFAP) or S-100 protein and subjected to autoradiography. Thereafter, locations of cells labeled autoradiographically and immunopositive for GFAP (GFAP+) or S-100 protein (S100+) were recorded. The examination revealed that the total number of autoradiograhically labelled astrocytes was maximal following [^{3}H]-thymidine injections on the 1st day after injury. However, patterns of changes in the number and distribution of autoradiographically labeled GFAP+ and S-100+ astrocytes displayed significant differences. Proliferating GFAP+ astrocytes were concentrated around the bottom part of the lesion whereas the distribution of proliferating S100+ astrocytes was much more diffuse. The results show that the brain injury inflicted within the early postnatal period leads to a permanent increase in the number of astrocytes. However, immunocytochemical stainings using different astrocyte markers present dissimilar patterns of astrocyte proliferative response to injury.

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A COMPARISON OF REACTIVE PROLIFERATION PATTERNS OF ASTROCYTES IMMUNOSTAINED FOR DIFFERENT MARKERS IN THE BRAIN OF ADULT MICE

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At different intervals following the injury of the cerebral hemisphere, a single dose of [^{3}H]-thymidine was injected and animals were killed 4 hours after the injection. Thereafter, the number and distribution of astrocytes proliferating within the injury area were recorded during microscopical observations of autoradiograms of brain sections immunostained for GFAP or for S-100 protein.

Quantitative analysis has revealed significant increase in the number of proliferating S-100 protein-positive (S-100+) astrocytes appeared earlier than that of GFAP-positive (GFAP+) astrocytes. The number of proliferating S-100+ astrocytes reached its maximal value on postoperative day 2 whereas the number of GFAP+ astrocytes was maximal on day 4, i.e. two days later. Proliferating S-100+ astrocytes were located closer to the injury site while GFAP+ astrocytes were distributed more diffusely.

It could be concluded that immunostainings recognizing different astrocyte markers revealed different distributions of astrocyte reactive proliferation.

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DISRUPTION OF THE SEPTOHIPPOCAMPAL PATHWAY LEADS TO ASTROGLIOSIS IN THE STRUCTURES DIRECTLY UNAFFECTED BY EXPERIMENTAL INTERVENTION

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We have previously demonstrated that the unilateral deafferentation of the hippocampus by means of the transection or electrolytic disruption of the septohippocampal pathways leads to the bilateral astrogliosis in the source of innervation - the septum, and in the target structure - the hippocampus. We are now reporting that such lesion causes the astrocytic reaction also in brain structures that are not directly affected by the experimental injury, mainly in portions of the thalamus and cortex. The astrocytic alterations were revealed as an increase in GFAP immunostaining and more than two-fold elevation of the number of GFAP-positive cells. In contrast to the reaction in the septum and hippocampus the astrocytic changes in the thalamus and cortex were limited to ipsilateral structures. Taken together, the differential remote responses of astroglia to brain damage, suggest their different susceptibility to factors possibly responsible for such reactions, like diffusable factors or various neuron-dependent signals.

VOLTAGE-GATED ION CHANNELS IN TROUT OLIGODENDROCYTES: BIOPHYSICAL AND MOLECULAR PROPERTIES

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Oligodendrocytes from the mature trout brain exhibit voltage-sensitive potassium outward currents, which consist of an inactivating component, resembling the A-type current and a steady-state component corresponding to a delayed rectifyer. Using the attached patch configuration a single channel conductance of 35-37 pS was determined for the delayed rectifyer current. To allow for a more precise characterization of the underlying ion channel protein the cDNA for the glial potassium channel was isolated by PCR-amplification of reversely transcribed mRNA extracted from trout glial cultures and subsequently sequenced. The deduced amino acid sequence of the 930 bp PCR product thus obtained exhibited a very high sequence homology with the mammalian delayed rectifyer potassium channel (RCK or shaker type channel) comprising six transmembrane helices. The highest degree of sequence identity was established in the S4-segment which is believed to contain the voltage sensor of the ion channel. Major differences occured in the extracellular loop connecting the S1 and the S2 transmembrane segment.

Oligodendroglial progenitor cells isolated from the larval trout brain by a novel immunomagnetic separation technique additionally displayed voltage-gated sodium currents. Regarding kinetics and pharamcology this current closely matched those of mammalian O2-A cells. During in vitro maturation of trout glial precursor cells the amplitude of the sodium current gradually decreased to became undetectable after seven days, when most of the cells had started to express the myelin glycoprotein IP2.

PROGESTERONE, A SEX STEROID HORMONE, INCREASES THE EXPRESSION OF MBP AND CNPase IN RAT OLIGODENDROCYTES IN PRIMARY CULTURE

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Primary cultures of rat glial cells were established from newborn rat forebrains. A mixed population of oligodendrocytes and astrocytes was obtained, as confirmed by indirect immunofluorescence staining with specific markers for each cell type. These glial cells contain progesterone, glucocorticosteroid, estrogen and androgen receptors (PR, GR, ER and AR), and only PR was inducible by estrogen treatment of the cultures (I. Jung-Testas, M. Renoir, J.M. Gasc and E.E. Baulieu, Exp. Cell. Res., 193, 1991). Different effects of progesterone (P) and estradiol (E_2) on cell multiplication, morphology and differentiation were observed during primary culture (I. Jung-Testas, M. Renoir, H. Bugnard, G.L. Greene and E.E. Baulieu, J. Steroid Biochem. Mol. Biol., 41, 1992). In this study we describe the effect of P on the expression of MBP (Myelin Basic Protein) and CNPase (2', 3'-cyclic nucleotide 3'-phosphodiesterase) during the first 10 days of primary culture. P (500 nM) slightly increased the number of MBP- and CNPase expressing oligodendrocytes, as does insulin (4.2 µg/ml). However, the combined application of P and insulin promoted a strong synergistic stimulation of the myelin proteins.

ULTRASTRUCTURAL ANALYSIS OF TRANSCELLULAR LABELLED MICROGLIA AFTER PHOTOCONVERSION WITH A CLOSED CONVERSION CHAMBER

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After axotomy of the mammalian optic nerve, retrograde labelling of retinal ganglion cells with the fluorescent dye 4Di-10ASP revealed that cellular debris of dying ganglion cells is phagocytosed by activated microlgia (Thanos, Europ. J. Neurosci. 3:1189-1207 (1991); Thanos et al., Exp. Eye Res. 55:101-117 (1992)). Photoconversion of the fluorescent dye and subsequent ultrastructural analysis confirmed that microglial cells incorporated 4Di-10ASP-labelled phagosomes (Seeger et al., Europ. J. Neurosci., Suppl. 6, p.283 (1993); Thanos et al., Trends Neurosci., (1994, in press)). In the present study we attempted to improve the photoconversion procedure by employing a closed conversion chamber in which the photoconversion is conducted in the presence of pure oxygen. As a result, in comparison with the photoconversion in an open petri dish, the application of pure oxygen in a closed conversion chamber reduces the appropriate time of illumination up to 50%. Due to the decreased exposure time, the background staining is very low and the number of successive conversions with one and the same specimen can be increased. Furthermore, by using a closed conversion chamber any emission of DAB which is supposed to be a carcinogenic substance is avoided.

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SEASONAL CHANGES IN ASTROCYTIC DYE-COUPLING WITHIN THE VOCAL CONTROL AREA HVC OF ADULT CANARIES

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We investigated the presence and the distribution of astrocytic communication and seasonal changes of astroglial interactions in the vocal control area HVC of male canaries by means of dye-coupling which was visualized by intracellular injection of neurobiotin into electrophysiologically identified astroglial cells. Double staining of dye-labeled cells with the astroglial marker GFAP verified the astrocytic nature of most of the dye-coupled cells. The overall spread of dye within the astroglial "syncytium" was comparable in both test groups (autumn versus summer), as well as within and outside HVc. However, the dye-coupling appeared to be reduced at the border of the HVc, indicating a partial functional segregation of astrocytic subpopulations within HVc and the surrounding tissue. In the HVc the total number of dye-coupled cells/mm^3 increased from 14241 (±791) during autumn to 22507 (±1498) during summer. This corresponds to functional aspects of the nucleus, as the animals reorganize their song production and repertoir during early spring. The data indicate that the astroglial population undergoes functional changes paralleling the seasonal plasticity of the vocal control nucleus HVc in the adult brain. The observed increase in the number of dye-coupled cells may reflect 1) an increase in the overall population of astrocytes during summer (gliogenesis), or 2) an additional recruitment of cells not beeing coupled in autumn.

INCREASED EXPRESSION OF THE GROWTH ASSOCIATED PROTEIN GAP-43 IN THE MYELIN-FREE RAT SPINAL CORD.

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The regional expression of GAP-43, a marker protein for fiber growth and plasticity, is mostly inverse to the pattern of myelination in the CNS of normal adult rats. Since myelin contains proteins with a strong inhibitory effect on neurite growth, these inverse expression patterns would be compatible with a role of neurite growth inhibitory proteins from oligodendrocytes in suppressing sprouting and synapse rearrangement in the normal mature CNS (Kapfhammer and Schwab, J.Comp.Neurol., *in press*). In order to study the relation between myelination and GAP-43 expression more directly, we have studied GAP-43 expression in the lumbar spinal cord of rats where myelination was suppressed by neonatal X-irradiation. In these spinal cords the typical regional downregulation of GAP-43 expression (as seen with GAP-43 immunohistochemistry) failed to occur and GAP-43 expression remained high in most parts of the myelin-free spinal cord. The increase in the total amount of GAP-43 in myelin-free spinal cords was confirmed by Western blot analysis. Non-irradiated, myelinated parts of the spinal cord of the same rats show the normal developmental suppression of GAP-43. These results support the hypothesis that myelin and neurite growth inhibitors suppress sprouting in the normal rat CNS.

FAST INHIBITION OF INWARDLY RECTIFYING K^+ CHANNELS BY NEUROTRANSMITTER RECEPTORS IN OLIGODENDROCYTES

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One of the essential functions of myelinating oligodendroglia in the mammalian central nervous system is the regulation of extracellular potassium levels by means of a prominent inwardly rectifying K^+ current. Cardiac and neuronal K^+ inward rectifiers are either activated by hyperpolarizing voltages or controlled by neurotransmitters through the action of receptor-activated G proteins. Neuromodulation of inward rectifiers has not been considered yet as a way to regulate oligodendrocyte function. Here we report the expression of serotonin, somatostatin, muscarinic acetylcholine and possibly further G-protein coupled receptors in cultured rat brain oligodendrocytes. Activation of these receptors leads to a partial or complete inhibition of Ba^{2+} and Cs^+ sensitive voltage-dependent inwardly rectifying K^+ channels, mimicked by intracellular perfusion with GTPγS (100µM). Onset of inhibition was fast with time constants τ_{ON} of 315±217 ms and relaxed with a slower offset (τ_{OFF} = 3608±960 ms) upon removal of the agonists. In heart and neurons the same receptors control a similar conductance in a diametrically opposed way. Substance P receptors which in CNS neurons inhibit anomalously rectifying K^+ channels were seen to mediate an increase in the amplitude of K^+ inward currents in oligodendrocytes.Thus, transmitter-mediated blockade and activation of inward rectifiers appears to be an oligodendrocyte-specific variation of a common motif for convergent signaling pathways. In vivo, expression of this mechanism which may be dependent on neuron-glia signaling may have a regulatory and so far unknown role in K^+ homoeostasis during neuronal activity in the CNS.

CYTOCHROME P450 IN CULTURED RAT ASTROCYTES - SUBSTRUCTURAL LOCALIZATION AND INDUCTION BY PHENYTOIN

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We investigated the cellular distribution of the inducible enzyme Cytochrome P450IIB1,2 in the rat brain and in astrocyte cultures derived from newborn rats. Astrocytes show immunoreactivity for Cytochrome P450IIB1,2 both in vivo and in vitro. Using immuno-electron microscopy in an preembedding technique we found peroxidase reaction products on distinct parts of the intracellular membrane systems i.e. the endoplasmic reticulum and preferably the outer membrane of mitochondrial and nuclear envelopes. Generally Cytochrom P450IIB1,2 is known to be a microsomal isoenzyme. On the level of light microscopy we found the immunoreactivity of astrocytes preferably in the elder brain parts and in close relationship to vessels and the meningues. In astrocyte cultures prepared from different brain parts, however, we did not observe such significant differences. The percentage of strongly immunoreactive astrocytes decreased from about 40% on day 7 in culture to about 15% after three weeks. In the same period of time nearly all astrocytes developed a weak, but significant immunoreactivity for P450IIB1. The administration of the anticonvulsant drug phenytoin in a concentration of 1µmol/l to the culture medium significantly (1.22 fold) increased the amount of total P450 in cell homogenates from the astrocyte cultures as measured by means of reduced CO-difference spectroscopy. Parallel to this result we found considarably more (1.5 fold) immunoreactive astrocytes in treated cultures than in controls.

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THE GLIAL NA^+-HCO_3^- COTRANSPORT IN THE CNS OF THE LEECH IS ACTIVATED BY THE PHORBOLESTER TPA

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In CO_2/HCO_3^--buffered solutions the electrogenic Na^+-HCO_3^- cotransporter is the major pH_i-regulating system in the glial cells of the medicinal leech *Hirudo medicinalis*. Previous experiments have shown, that when a HEPES-buffered solution was exchanged for a solution buffered with 2 % CO_2/11 mM HCO_3^- the mean steady-state pH_i of the glial-cells increased from 6.85 to 7.1 and the membrane potential hyperpolarized by 8-12 mV(1)
We investigated the effect of the phorbolester TPA (12-O-Tetradecanoylphorbol-13-acetate) on the pH_i-change in CO_2/HCO_3^- buffered solutions using double-barreled pH-sensitive microelectrodes in neuropile glial cells of the medicinal leech.
We found, that after the application of TPA the rate of the CO_2/HCO_3^- induced pH_i-change increased significantly by 66.8 ± 40.0 % (paired t-test; $p < 0.05$;n=6). The rate of the pH_i-change was calculated from an exponential fit of the pH-trace.
In addition the steady-state pH_i increased by 0.05 ± 0.07 pH-units (n=5, $p<0.1$) and the membrane hyperpolarization increased by 36 ± 34.5 % (n=24, $p<0.1$)).
We conclude that the glial Na^+-HCO_3^- cotransport in the CNS of the medicinal leech is activated by the phorbolester TPA

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PROTOPLASMIC ASTROCYTES WHICH HAVE CONTACTS TO BLOOD VESSELS AND SYNAPSES DISPLAY NMDA-EVOKED INWARD CURRENTS

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Recent evidence suggests that glial cells form complex, functional interactions with synaptic regions. In particular, in the neocortex glial cells contact neuronal cell-bodies, which are enwrapped by a net-like structure of extracellular matrix components, called perineuronal nets of matrix.
To study the physiological properties of such specialized cells, we applied the patch-clamp technique to characterize the membrane properties of glial cells in a thin slice preparation of the parietal and cingular neocortex from three-week-old mice. To identify the glial cells, we combined the patch-clamp technique with morphological analysis by dialysing the cell with a pipette solution containing fluorescent dyes. The dye was either photoconverted using the diaminobenzidine method for ultrastructural analysis, or it was visualized by confocal microscopy. Different types of glial cells could be investigated. In this study, the physiology, morphology and cellular environment of a particular subtype was examined in detail. These cells investigated had small somata (diameter less than 10 µm), their processes were densely arborized and the distal endfeet contacted blood vessels, thus identifying these glial cells as protoplasmic astrocytes. In addition, more proximal processes enwrapped synapses on the soma region of neighbouring neurons. But, they did not express the glial fibrillary acidic protein. The membrane potential of these astrocytes ranged between -60 to 65 mV and de- or hyperpolarizing voltage steps activated large, time-independent K^+ currents with a linear current to voltage relationship. Strikingly, NMDA, as a glutamate receptor agonist, elicited inward currents up to more than 700 pA, which could be blocked by the specific antagonist MK801. These receptors which are known to be involved in signal transduction could provide the molecular substrate for a signal transfer from the synaptic region to the adjacent glial cell. Contact sites with neuronal cell surfaces as well as with blood vessels, the expression of gluatamate receptors of the NMDA subtype and a high K+ conductance might endow this type of astrocyte with a particular integrative function in sensing and modulating neuronal activity.

ATP-induces cytoplasmic Ca^{2+} release via activation of $InsP_3$-sensitive channels in oligodendrocytes.

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The subcellular characteristics of ATP-induced elevation in cytoplasmic calcium concentration ($(Ca^{2+})_{in}$) were studied in cultured cells of the oligodendrocytic lineage obtained from mouse cortex and retinae as well as of oligodendrocytes in corpus callosum slices by the use of laser confocal scanning microfluorimetry. Cells were loaded with fluo-3 by either incubation with acetomethylester form or via the patch pipette. The developmental stage of cultured oligodendrocytes was defined by immunostaining with the O4 and O10 antibodies. Bath application of ATP in micromolar concentrations induced a transient increase of $(Ca^{2+})_{in}$ in immature and mature oligodendrocytes but not glial precursor cells from retinal and cortical culture and from corpus callosum slices. The ATP-induced $(Ca^{2+})_{in}$ elevation was significantly higher in oligodendrocyte processes as compared to the soma. The simultaneous transmembrane current recordings as performed in a whole-cell patch clamp configuration did not reveal any changes in membrane conductance associated with the ATP application. Similar ATP-induced Ca^{2+} transients were observed also in Ca^{2+}-free extracellular solution, suggesting that ATP-mediated elevation of $(Ca^{2+})_{in}$ is due to calcium liberation from intracellular stores. The rank order of potency for the tested purine and pyrimidine nucleotides was: UTP ≥ ATP > ADP >> AMP = Adenosine = Me-ATP for retinal oligodendrocytes and: ADP ≥ ATP >> UTP = AMP = Adenosine = Me-ATP for cortical oligodendrocytes. The response to ATP and related nucleotides could be reversibly inhibited by the P_2 purinoreceptor antagonist suramin. ATP-induced elevation of the cytosolic Ca^{2+} concentration was abolished by cell incubation with thapsigargin (10 μM) and by intracellular administration of heparin (10 μM). These findings indicate that ATP triggers a release of Ca^{2+} ions from $InsP_3$-sensitive internal stores. The $InsP_3$-sensitive calcium stores in oligodendrocytes, once depleted of Ca^{2+} by ATP exposure, had an absolute requirement for extracellular calcium to refill; this refilling was achieved presumably due to Ca^{2+}-ATPases dependent Ca^{2+} sequestration by endoplasmic reticulum. The ATP receptors may play a role in neuron-glial interactions, in particular under pathological conditions when ATP might be released from damaged cells.

ATP triggers Ca^{2+}mobilization in Bergmann glial cells via activation of P_2 purinoreceptors

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The property of ATP receptors was studied in Bergmann glial cells from thin cerebellar slices of mice during different stages of development. The whole cell patch-clamp technique was used to load individual Bergmann glial cells with a Ca^{2+} probe, fluo-3. The agonist -induced elevation of the cytoplasmic calcium concentration ($[Ca^{2+}]_{in}$ was monitored by means of laser confocal scanning microfluorimetry. ATP induced an transient elevation of $[Ca^{2+}]_{in}$ which originated from processes and spread towards the cell soma. In contrast to astrocytes and microglial cells, ATP did not induce a conductance increase in the Bergmann glial cells. The Ca^{2+} elevation was mediated by an intracellular release since the response was still observed in Ca^{2+}-free bathing solution. The response was mediated by a P_2 purinoreceptor since the antagonist suramin blocked the response and since we found the following rank order of potency: ADP > ATP > UTP >> AMP = adenosine = Me-ATP. The ATP mediated Ca^{2+} response was found in all cells from adults, but only in about half of less mature Bergmann glial cells (P16 to P20). ATP receptors represent thus an additional system (besides GABA, kainate and NMDA receptors) by which Bergmann glial cells can respond to external signals.

Subcellular heterogeneity of voltage-gated Ca^{2+} channels in cells of the oligodendrocyte lineage

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We studied the subcellular topology of voltage gated Ca^{2+} transients in cells of the oligodendrocyte lineage from retinal and cortical cultures loaded with the intracellular calcium probe fluo-3. Influx of Ca^{2+} via voltage-gated channels was activated by membrane depolarization with elevated $[K^+]$ and local, subcellular increases in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{in}$) could be monitored with a fluometric system connected to a laser scanning confocal microscope. In glial precursor cells from both retina and cortex, small depolarizations (with 10 or 20 mM K^+) activated Ca^{2+} transients in processes indicating the presence of low-voltage-gated Ca^{2+} channels. Larger depolarizations (with 50 mM K^+) additionnally activated high-voltage-gated Ca^{2+} channels in the soma. Ni^{2+} (50μM), a selective blocker of low-voltage gated Ca^{2+} channels, strongly inhibited Ca^{2+} transients in processes, but not in the soma. An uneven distribution of Ca^{2+} channels was also observed in the mature oligodendrocytes in that Ca^{2+} transients in processes were considerably larger as compared to the soma. Recovery of $[Ca^{2+}]_{in}$ after the voltage-induced influx was achieved by the activity of the plasmalemmal Ca^{2+} pump, while mitochondria played a minor role in buffering $[Ca^{2+}]_{in}$ elevated due to influx through voltage-operated channels. During the development of white matter tracts, cells of the oligodendrocyte lineage contact axons to form myelin. Neuronal activity is accompanied by increases in extracellular $[K^+]$; this may lead to a $[Ca^{2+}]_{in}$ increase and might represent a signal for the glial precursor cell to start myelin formation.

STRATEGY TOWARDS A LOCALIZATION OF ADENYLATE-DEAMINASE IN RAT BRAIN

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As part of the purine nucleotide cycle (PNC) adenylate-deaminase (AMP-DA) catalyzes the hydrolytical deamination of AMP to IMP thereby releasing the neurotoxic NH_4^+. The physiological function in brain of the PNC is still unknown. We consider a role in association with amino acid degradation in the brain. Thus, we propose transaminations of the amino group via glutamate to oxalacetate resulting in aspartate that delivers the amino group of amino acids into the PNC in an irreversible reaction. In addition we hypothezise colocalization of AMP-DA with the NH_4^+-metabolizing glutamine synthetase in astroglial cells.

In an effort to cellularly allocate AMP-DA in the brain a new efficient method of purification has been developed for the AMP-DA from sheep brain. The enzyme will serve as an antigen for the production of antibodies that are to be used in immunohistochemical studies. The allocation is also approached by in situ hybridization using antisense cDNA probes for AMP-DA mRNA.

RELATIONSHIP BETWEEN ASTROGLIAL ALTERATIONS AND NEURONAL EVENTS IN TRIMETHYLTIN TREATED RATS.

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A single systemic administration of trimethyltin, an environmental neurotoxin, induces reactive gliosis (monitored by immunocytochemistry for glial fibrillary acidic protein, GFAP) and specific changes to the dendrites (mapped by immunocytochemistry for microtubule-associated protein 2, MAP-2) in the hippocampus. While an increase in GFAP immunoreactivity (-IR) accompanies a decrease in MAP-2-IR at the sites of neuronal injury (CA4/CA3 and CA1 hippocampal subfields), a concomitant increase in immunostaining of both markers is found in the area denervated by the lesion (i.e. in the molecular layer (ML) of dentate gyrus). It has to be noted that in our conditions reactive astrocytes do not express MAP-2, in opposite to the situation reported by others after a stab wound lesion. Instead, astroglial cells are immunostained for nerve growth factor. We suggest that dendritic changes in the ML may reflect granular cell response to denervation/reinnervation processes, while astrocytes may contribute to these phenomena passively by removing degenerating debris and/or actively by producing neurotrophic factors which, in turn, may favor specific cytoskeletal changes in postsynaptic elements.

STIMULATION OF CULTURED ASTROCYTES WITH LIPOPOLYSACCHARIDES OR LEUKOTRIENE B4: COMPARISON OF ELECTROPHYSIOLOGICAL AND IMMUNOLOGICAL EFFECTS

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After stimulation with immunologically active substances like leukotriene B_4 (LTB_4) and lipopolysaccharides (LPS) electrophysiological properties of cultured astrocytes from new-born rats were tested in order to investigate a coupling between immunological activation and putative functional impairment. Both substances lead to a marked depolarization. In case of LTB_4 (1 µM) this depolarization could be mimicked by the addition of Ba^{2+} (2 mM) and was not increased by the application of LTB_4 and Ba^{2+}, indicating a similar mechanism inducing the depolarization, e.g. a reduction of K^+ conductance. In contrast, the depolarization induced by LPS (5 µg/ml) was abolished in Na^+ - free solution and was blocked by amiloride (250 µM), both providing evidence for an activation of a Na^+ dependent transport system as a source for the depolarization. Cultured astrocytes could be immunologically activated to release IL-6 in response to application of LPS (10 µg/ml) but not in response to LTB_4.

Our results indicate that both substances lead to a marked alteration of electrophysiological properties by specific intracellular mechanisms and the depolarization is not a crucial stimulus for the production of IL-6. However, the marked depolarization, which e.g. impairs the glial capacity for maintaining ional homeostasis, may contribute to neuronal and glial dysfunction during inflammatory CNS diseases.

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NEUROSTEROIDS : PREGNENOLONE AND PROGESTERONE PROMOTE MYELINATION IN REGENERATING PERIPHERAL NERVE

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The neurosteroids pregnenolone (PREG) and progesterone (PROG) have been shown to be synthetized *in vitro* by Schwann cells (SC), the latter only in presence of DRG neurons (see poster M. Schumacher). *In vivo*, mouse sciatic nerves contain high levels of PREG and PROG as compared to plasma.

To determine if nerve endogenous neurosteroïds are involved in the process of PNS myelination (as suggested by their low levels in Trembler hypomyelinating mutant), sciatic nerves of male mice were frozen and subsequently supplied by 3 external applications of Trilostane (inhibitor of PROG synthesis) and/or RU 486 (antagonist of PROG). With both compounds, the mean thickness of the neoformed myelin sheaths was decreased by 35 and 27% respectively. In the same experimental system, local applications of exogenous PREG and/or PROG at high concentrations increased by approximatly 20% the average thickness of the myelin sheaths.

The effects of the steroids and inhibitors on PNS remyelination was markedly evident when the regenerating myelin sheaths were classified according to the number of lamellae formed.
As a result, the number of thick myelin sheaths was increased by steroids and decreased by PROG inhibitors, whereas the reverse was obtained for the thin myelin sheaths.

ASTROCYTES EXPRESS BIGLYCAN IN VITRO

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Cultured astrocytes from newborn rats express a set of diverse proteoglycans. Starting from astrocyte-conditioned medium (ACM) a small chondroitin-sulfate proteoglycan (Mr 200-300kD) was highly enriched using two anion exchange chromatography steps (native Q-Sepharose- followed by denaturing MonoQ chromatography). A significant portion of the neuronal survival-enhancing activity of ACM co-purified with the proteoglycan. Digestion with chondroitinase ABC revealed a major core glycoprotein band (Mr 50 kD) identical in size to the biglycan core glycoprotein from a different source. Northern analyses and *in-situ* hybridization proved that astrocytes express biglycan mRNA. Biglycan is a small leucine-rich proteoglycan of the extracellular matrix that was highly conserved during evolution. Its function is unknown yet. Our current investigations focus on (a) the relationship between survival-enhancing activity and biglycan expression, (b) regulation of biglycan mRNA expression in astrocytes by TGF-ß and (c) expression of biglycan mRNA in the nervous system.

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GROWTH FACTORS AND THE RADIATION RESPONSE OF OLIGODENDROCYTE - TYPE 2 ASTROCYTE (O-2A) PROGENITOR CELLS.

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The purpose of this study was to assess the effects of growth factors on (1) their ability to stimulate the proliferation of O-2A cells, and (2) their potential to modify the radiation-induced damage in these progenitor cells. Cultures of dispersed cells from spinal cords of perinatal Wistar rats were used. The fraction of O-2A cells in these cultures was 25%. Using bFGF/PDGF treatment this percentage was increased to more than 50%. Cell proliferation was measured by ^{3}H-Thymidine incorporation. The potential to proliferate was measured by clonogenic assay. From the assayed growth factors (CSF, EGF, bFGF, NGF, PDGF, SCF) bFGF and PDGF had the strongest mitogenic stimulation. bFGF had the most predominant effect on the proliferative response after irradiation. Especially after the higher irradiation doses (6 and 8 Gy), cultures treated with this growth factor incorporated more ^{3}H-TdR and formed more (small) colonies in the clonogenic assay. In contrary to bFGF and PDGF, only EGF stimulated proliferation in purified type 1 astrocytes. However, neither EGF nor bFGF showed a radiation-protective effect. The effect of growth factors on enriched cultures of type 2 astrocytes and oligodendrocytes are presently under investigation.

STRAIN DEPENDENT EXPRESSION OF TNF-α AND MAJOR HISTOCOMPATIBILITY COMPLEX BY RETINAL GLIAL (MÜLLER) CELLS.

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Retinal S-antigen (S-Ag)-induced experimental autoimmune uveoretinitis (EAU) and endotoxin-induced uveitis (EIU), models for human ocular immunopathological syndromes, result in ocular inflammation and rapid photoreceptor degeneration in susceptible, but not in resistant strains of rat. The retinal Müller glia cell (RMG) were shown to play a role in local ocular immune regulation, suppressing the proliferative response to S-Ag of S-Ag-specific T cell lines in the presence of antigen presenting cells and in certain conditions to function as antigen-presenting cells. In order to determine whether local immune response could account for differences in resistance or susceptibility, these cells were isolated and cultured *in vitro* from Lewis (highly susceptible), Brown-Norway (BN) (poorly susceptible), Lewis x BN F1 hybrid (susceptible) and Long-Evans (LE) (refractory) rats. MHC class II Ag expression was strongly induced by combined IFN-γ and LPS treatment in RMG cultured from Lewis, variably from BN, and only weakly from LE rats. However, increased MHC class II Ag expression by LE RMG could be induced with more potent stimulation (supernatant from Concanavalin A-stimulated rat splenocytes). Correspondingly, constitutive production of the cytokine TNF-α, or its liberation in response to either IFN-γ or LPS alone, was very low in RMG cells, irrespective of strain. It was strongly induced by combined treatment with IFN-γ and LPS in Lewis RMG cells (mean values of 140 pg/10^5 cells) and in Lewis x BN F1 RMG cells (mean values of 110 pg/10^5 cells), much less so in BN RMG cells (30 pg/10^5 cells) and remained undetectable in LE RMG cells. Hence susceptibility to EAU and EIU is correlated with the extent of TNF-α production by this ocular resident cell which may play a key role in initiating or perpetuating local immune responses.

OPIOID PEPTIDES AS GROWTH FACTORS FOR GLIAL CELLS OF PNS, CNS. MODEL IN CULTURE

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Opioid peptides (OP) (endogeneous and leu-enkephalin analogue - dalargin) were found to decrease the proliferation of CNS glial cells as testified by a decrease (37%) in the number of cells and by inhibition (35%) of DNA synthesis, to decrease proliferation and to increase migration ability of PNS glial cells, to increase the survival and adhesion by formation of cellular aggregates of spinal cord (the area of aggregates was increased an average by 1,6-1,7 fold. Glycerophosphate dehydrogenase activity in Schwann cells and CNS glia increased under action of OP that exceeded the control value 2,2 and 2,1 fold respectively. OP decreased proliferation and DNA synthesis of C-6 glioma in culture medium with big % serum but increased its phenotype expression in early and late passages on cytochemical screening.
Thus OP increased not proliferation but increased very actively differentiation and change the adhesion of glial cells.

O-2A PRECURSOR CELL LINES GENERATED FROM PRIMARY CELLS IMMORTALISED WITH A REGULATEABLE NEU TYROSINE KINASE GENE RETAIN PHYSIOLOGICAL PROPERTIES *IN VITRO* AND *IN VIVO*

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Cultures of murine oligodendrocytes and their precursor cells were infected with a replication-defective retrovirus expressing a hybrid gene coding for the extracellular domain of the human EGF receptor and the intracellular domain of the neu tyrosine kinase. An O-2A precursor cell line (Oli-egfr-neu) was established, from which three clones derived via single cell cloning were characterised. In the presence of EGF the cells continually proliferated and in morphology and antigenic phenotype they resembled normal murine O-2A cells with most cells staining with the LB1 antibody. Withdrawal of EGF from the culture medium lead to a reduced proliferation and limited differentiation to a more mature phenotype followed by cell death after 7 to 10 days. Electrophysiological studies showed that most of the cells expressed the kainate subtype of glutamate receptors and some of the cells expressed $GABA_A$ receptors. When transplanted into demyelinated lessions in the adult rat spinal cord with no intrinsic repair capacity, some cells ensheathed the demyelinated axons in an oligodendrocyte-like manner and expressed the myelin-associated glycoprotein. Other cells were stained with antibodies to GFAP and associated with blood vessels. The cell line and the clones both retain physiological properties and are thus useful tools for investigating cell-surface antigens involved in regulating glial cell function.

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EXPRESSION PATTERN OF A BLOOD-BRAIN SPECIFIC ENZYME IN ACUTE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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The experimental autoimmune encephalomyelitis (EAE) serves as a model for multiple sclerosis with episodes of paralysis and a focal breakdown of the blood-brain barrier (BBB) followed by clinical remission. The transient opening of the BBB occurs in an extravasation of cytotoxic T-lymphocytes and macrophages (perivascular infiltrate) and an increased BBB-permeability to serum proteins.

The structural changes of the BBB-components during the inflammatory reaction have been described but exact molecular and enzymatic deficiencies are not well defined.

We have examined the expression pattern of the BBB-specific pericyte-associated enzyme aminopeptidase N (pAPN) and we found a good correlation with the functional BBB-breakdown in acute EAE. The occurrence of perivascular infiltrates is associated with a significant decrease of vascular-associated pAPN. Cerebral function of pAPN has been discussed as a neuropeptide degradating enzyme. The lack of pAPN in acute EAE could be involved in the metabolic opening of the BBB and allow entry of blood-borne neuropeptides e.g. enkephalines, substance P.

At day 6 after EAE induction the vascular pAPN-expression was maximally reduced. In contrast many immunochemical pAPN positive cells, apparently invading the parenchyma from the perivascular space could be observed especially in the white matter of the lumbal spinal cord. The invasive pAPN positive cells also express marker proteins for activated macrophages (ICAM 1 and Ox 42). The migration of perivascular cells into cerebral parenchyme is facilitated by an enhanced vascular expression of pAPN at day 1 to 4 of EAE. The enhanced expression is interpreted to allow the initial degradation of basement membranes for the migratory onset. Cerebral pericytes can thus be interpreted as "silent macrophages".

MOLECULAR CLONING OF RAT INTERLEUKIN-1 RECEPTOR ANTAGONIST: REGULATION OF EXPRESSION IN THE LESIONED SCIATIC NERVE

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We have previously shown that transection of the rat sciatic nerve results in a biphasic mode of increase in NGF- mRNA in Schwann cells and fibroblasts surrounding the axons at the tip of the proximal stump and distally to the site of injury (1). The rapid transient increase within hours is dependent on the stimulation of c-fos and c-jun (2) while the second and persistant rise in NGF-mRNA results from the invasion of macrophages (3) synthesizing and releasing interleukin-1 (4) . In order to be able to dissect between macrophage dependent and independent effects of interleukin-1 we study the physiology of interleukin-1 receptor antagonist protein (IRAP). Although IRAP- mRNA levels are barely detectable in the intact nerve, its levels are strongly increased after lesion in a time course , that is slightly delayed as compared to the initial rise in NGF- mRNA.We have isolated and characterized the cDNA of rat IRAP. There was an 88 % overall identity between rat and mouse IRAP at the level of amino acids and a 92,3 % identity based on the nucleotide sequence. Together with results obtained from the application of recombinant rat IRAP in the cultured sciatic nerve we conclude that endogenous IRAP expression may influence the NGF- dependent peripheral nerve regeneration.

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Developmental changes in the expression of K^+ and Na^+ channels in glial cells of hippocampal slices

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Glial cells in grey matter of the CNS can be distinguished according to their distinct electrophysiological properties. Based on their typical pattern of membrane currents four populations of glial cells could be identified in thin brain slices of the postnatal hippocampus. In the present study properties of voltage gated K^+ and Na^+ channels were analyzed at different stages of cell maturation during postnatal development. We focused on 'complex' cells which presumably represent a population of glial precursor cells.

Membrane currents were characterized in situ with the patch clamp technique. Cells were located in the stratum radiatum of the hippocampal CA1 subregion. At postnatal day (P) 5 to P12 large outwardly rectifying K^+ currents were activated by membrane depolarization. These currents were mainly composed of a transient and a sustained component which closely resembled properties of neuronal A-type and delayed rectifier K^+ currents. In contrast, in cells of P20 - P33 mice a time and voltage independent current developed while both voltage dependent components were down regulated. In addition to the appearance of passive currents, all cells at the late developmental stage expressed prominent inward rectifier K^+ channels which only rarely were detected at P5. The developmental regulation of transient glial Na^+ currents was similar to that of transient and delayed outward rectifier K^+ currents. The percentage of cells possessing significant Na^+ currents decreased from 100 % (P5) to about 30 % (P20-P25). We stained the cells of the CA1 stratum radiatum for glial fibrillary acidic protein (GFAP), a marker that recognizes mature astrocytes. Between P5 and P30 the changes in electrophysiological properties were accompanied by a threefold increase in the relative number of GFAP positive cells.

We conclude that the heterogeneity in electrophysiological properties of glial cells, as revealed in various areas of the juvenile CNS is at least partly due to the coexistence of variable stages of cell maturation at a given age.

SUBSTRATE PROPERTIES OF CNS MYELIN AND OLIGODENDROCYTES IN *XENOPUS* OPTIC NERVE DIFFER FROM THOSE IN SPINAL CORD

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In adult *Xenopus* (amphibia), retinal axons regrow after transection of the optic nerve but lesioned axons in the spinal cord fail to regenerate. To examine whether the substrate properties of oligodendrocytes and CNS myelin in the spinal cord differ from those in the optic nerve, fore- and midbrain, the interaction of axons with oligodendrocytes and CNS myelin was analyzed. Quantitative axonal outgrowth assays on myelin substrates and time lapse recordings of growth cone-oligodendrocyte interactions combined with immunocytochemical studies yielded the following results: In contrast to the situation in the optic nerve, forebrain and midbrain, myelin and oligodendrocytes of the *Xenopus* spinal cord are non-permissive substrates for axon growth and appear to exhibit the neurite growth inhibitors identified by M.E. Schwab and co-workers.

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SPECIES DIFFERENCES IN DOPAMINE D_1 AGONIST EFFICACY IN ASTROCYTES FROM ADULT RAT, MONKEY AND HUMAN BRAIN

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Recently, species differences were reported in the pharmacology of dopamine D_1 receptors, which are stimulatory linked to adenylate cyclase. This receptor type is present on cultured astrocytes. In the present study, we compared the efficacy of various selective dopamine D_1 receptor agonists in cultured astrocytes from adult rat, monkey and human brain.

For this purpose, we determined the increase in adenylate cyclase activity following incubation of the cells with the drugs. The efficacy of the D_1 agonists was expressed as a percentage of the maximal effect of the endogenous transmitter dopamine.

Our results show that SKF 38393 is a partial dopamine D_1 agonist in cultured rat, monkey and human astrocytes. In human and monkey astrocytes, however, the efficacy of SKF 38393 was much lower (7-13%) than in rat astrocytes (62%). SKF 81297 was a full efficacy D_1 agonist in rat astrocytes. In human and monkey astrocytes it was a partial agonist (efficacy 53% and 34% respectively). In contrast, A 68930 was found to be a full efficacy D_1 agonist in all species studied.

In conclusion, cultured astrocytes proved to be a suitable model to study the characteristics of dopamine D_1 receptors from various species (including humans). The results suggest that the classical D_1 agonist SKF 38393 is unsuitable as a tool to study dopamine D_1 receptor function in primates.

ASTROGLIAL REACTIONS LABEL INITIAL STAGES OF SYNAPTIC REORGANIZATION FOLLOWING FACIAL NERVE DYSFUNCTION IN ADULT RATS

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In adult rats, facial nerve lesions do not only induce synaptic reorganization in the facial nucleus after many hours ("synaptic stripping" on motoneurons; Blinzinger & Kreuzberg '68) but also modify the somatotopic representation map in the motor cortex. Electrophysiological studies indicate that the latter type of neuroplasticity is based on rapid ($\geq$ 45 min postoperative; p.o.) changes in the effectiveness of certain associational connections from facial nerve representation to neighbouring cortex zones and that transient modifications may lead to some long term consolidation process (Sanes et al '88).Here, we report on rapid reactions of astrocytes in both, the facial nucleus and the motor cortex, following facial nerve lesions (transection, crush) or other disturbances of its function (e.g.,Botulinum toxin injections into facial muscles to block synaptic transmission at motor endplates) in adult Sprague-Dawley rats.

In the ipsilateral facial nucleus, immunoreactivity (IR) for Connexin 43 (Cx43; the gap junction protein of astrocytes) significantly increases within the first hour after after injury and remains elevated for several days. This rapid reaction antedates by several hours the augmentation of GFAP-IR in astrocytes and synaptic stripping on motoneurons.

In the contralateral motor cortex, both, the IR for S100 protein (a Ca-binding protein predominantly located in astrocytes) rapidly increases in band-like cortex zones bordering the facial representation zone from about 1 hour p.o. onwards. This reaction of S100-IR is apparently transient. After more than 8 hours increased S100-IR shifts to the facial nerve representation zone, from where it also vanishes after about 2 days, even if nerve regeneration is prevented. Similar band-like patterns of increased IR were found for Cx43 shortly after lesioning and for GFAP after more than 24 hrs. Electron microscopical observation are compatible with the hypothesis that astrocytes monitor or are even involved in regulating synaptic plasticity on neighbouring neurons. This astroglial reaction apparently vanishes after consolidation of plastic changes.

MITOGENIC EFFECT OF VASOPRESSIN AND ALTERATION OF ION TRANSPORT IN CULTURED ASTROGLIAL CELLS.

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Primary cultures of astroglial cells were derived from cerebral cortices of newborn rat pups. After 8-10 and 27-30 days of culturing, respectively, the astroglial cells were exposed to arginine-vasopressin (AVP) for 10 min-120 hours. Incorporation of ^{3}H-thimidine, bumetanide or amiloride-sensitive uptake of $^{22}Na^{+}$ and $^{36}Cl^{-}$, as well as changes of cell number, protein- and DNA content in the cultures were subsequently assayed. In a dose-dependent manner (10^{-6} - 10^{-9} M) AVP increased both bumetanide- and amiloride-sensitive uptake of $^{22}Na^{+}$ and $^{36}Cl^{-}$ after 20-30 min of exposure. Within this concentration range AVP significantly increased ^{3}H-incorporation, cell number, DNA- and protein content in the cultures, however, after an exposure period longer than 24 hours. All of the AVP effects could be inhibited by the V_1 receptor antagonist Manning compound. Astroglial cells performed the maximum response against AVP after a 27-30 days long period of cultivation in serum containing culture medium.

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NITRIC OXIDE AS INTRACELLULAR MESSENGER FOR IFNγ STIMULATED ASTROCYTOMA CELLS

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Nitric oxide (NO) is an unstable nitrogen radical, spontaneously degradating into nitrites, which exerts its biological activity via the stimulation of the soluble guanylate cyclase. It is generated through different isoforms of NO synthase. Astrocytes have been reported to express both constitutive and inducible NO synthase (NOS) isoforms. IFNγ elicits immunoaccessory properties in these cells, as MHC class II antigen expression. Recently, we have demonstrated that in astrocytoma cells IFNγ induces the iNOS, leading to an increase in nitrite concentration in cell supernatants. Furthermore, L-NAME and methylene blue, inhibitor of NOS and NO biological activity respectively, reduce the IFNγ-induced MHC class II antigen expression, suggesting NO involvement in this mechanism.

An other effect mediated by IFNγ on these cells is the dose-dependent modulation of the synthesis and secretion of a protease inhibitor, $\alpha_2$macroglobulin, as evaluated by ELISA and PCR analysis. It has been reported that this factor may be important in the antigen presentation processes. Our preliminary data suggest that, in this case, NO may be involved in the intracellular messenger associated with IFNγ stimulation.

POSTNATAL DEVELOPMENT OF THE CIRCADIAN CLOCK IN THE GOLDEN HAMSTER : PUTATIVE ROLES OF ASTROCYTES.

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The fetal clock begins to oscillate prenatally and is entrained by maternal rhythm. In the adult, the circadian clock located in the hypothalamic suprachiasmatic nuclei (SCN) is entrained by the light-dark (LD) cycle. The photoperiodic signal received by the retina, directly reaches the SCN via the retino-hypothalamic tract (RHT). The neurotransmitter released by RHT terminals is likely to be glutamate. At birth retinal ganglion cells terminals do not reach the SCN. An indirect way to estimate the astrocytic function in glutamate uptake is to follow glial activity within pups SCN from birth until the clock can be entrained by light. This study was performed : *i*) to determine when the retina-SCN connection is established (anterograde tracing with cholera toxin) and become functional (induction of c-*fos* by light) and *ii*) to follow GFAP-immunoreactivity (Ir) in parallel to RHT development. Newborns from mothers entrained in an LD cycle were perfused at the same time of the 24h period at various postnatal days (PN1 to PN21 = weaning). Brain coronal sections were processed for immunohistochemistry. The synchrony between RHT development and c-*fos* induced response by light strongly suggests that as soon as fibers penetrate the SCN (from PN7) they form functional synapses. The parallel and progressive invasion of GFAP-Ir from PN4 to the adult-like pattern (PN13) indicates that astrocytes could be involved in the regulation of SCN extracelular glutamate released by the RHT. Before PN4, presumed age of complete SCN synaptogenesis (Speh & Moore, 94), synchronization of metabolic or electrical activity is observed. We hypothesized that this early cellular orchestration is supported by immmature astrocytes. In order to visualize their distribution, an anatomical study has been undertaken from birth, using vimentin-Ir.

INDUCTION AND REGULATION OF INTERLEUKIN-6 SYNTHESIS AND RELEASE IN NEWBORN MOUSE MICROGLIA

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It has been demonstrated that glial cells of central nervous system synthesize cytokines both *in vivo* and *in vitro* under lipopolysaccharide or interleukin-1 (IL-1) stimulation (VanDam *et al.* 1992 ; Merryl 1992). IL-1 receptors are present on astrocytes (Ban *et al.* 1993) and in mouse hippocampus (Parnet *et al.* 1992). Glucocorticoids are known to be potent inhibitory factors for cytokines synthesis and release (Staruch *et al.*, 1985) and to upregulate IL-1 receptors (Gottschal *et al.* 1991).

We first investigated the presence of IL-1 receptor mRNA in secondary cultured microglial cells provided from primary cultures of hypothalamic and hippocampal cells of newborn mice, using semi-quantitative polymerase chain reaction (PCR). In order to provide that glial cells could not only synthesize but also release cytokines after an immune stimulation, we investigated IL-1-induced interleukin-6 (IL-6) liberation by a reverse hemolytic plaque assay (RHPA) (Smith *et al.* 1984). RHPA coupled to immunocytochemical studies allowed us to identify the phenotype of the cells that released IL-6. Moreover, semi-quantitative Biocom analysis of hemolytic plaque area permitted us to compare constitutive release of IL-6 with the liberation of IL-6 induced by IL-1 and modulated by a short exposure to glucocorticoids. By this technique we will be able to study the release of cytokines in oligodendrocyte or astrocyte purified cultures.

By coupling PCR and RHPA studies we should further understand the mechanisms of central cytokine synthesis and release *in vitro*. These mechanisms are believed to play an important role in the establishment of the cytokine network, and are therefore implicated in the physiological and behavioral modifications occuring during infection.

ASTROCYTIC PLASTICITY IN NATURAL SCRAPIE OF THE SHEEP

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Subacute spongiform encephalopathy is a degenerative disease of the central nervous system. The precise aetiology is unknown. The causal pathogen is mainly a host normal protein (PrPc for cellular Prion Protein) which is transformed during the course of the disease in an abnormal protein (PrPsc for scrapie Prion Protein). PrPsc has been shown to accumulate in astrocytes and to be tightly associated with the glial fibrillary acidic protein (GFAP).

In order to better understand how astrocytes may be involved in the aetiology and/or development of the disease, we investigated in the natural scrapie of the sheep, the molecular expression of GFAP and glutamine synthetase (GS), protein of the astroglial metabolism. Four structures of the sheep brain have been choosen: cerebellum, pons, thalamus, and caudate nucleus. Northern and western blotting assays indicate: 1- an astroglial reactivity which occured in precise areas of the scrapie sheep brain. 2- a GFAP overexpression, transcriptionnaly regulated in the cerebellum and pons. 3- a GS overexpression, transcriptionnaly regulated in the thalamus. 4- a correlated increase in the GFAP and GS expression present only in cerebellum and pons of only a part of the scrapie sheep. 5- finally, an astroglial reactivity appearing early in the course of the disease, but unrelated to the severity of the disease.

These observations suggest that an astroglial dysfunction was present early in the natural scrapie of the sheep. This dysfunction was not generalized, but was located in some brain areas and might be related to particular neuronal and astroglial cells. It concerns at least a transcriptionnal regulation of the astroglial intermediate filament major component, and of a protein involved in the astroglial metabolism and in brain detoxification.

IONIC CHANGES AND DIFFUSION PARAMETERS IN CNS DURING DEVELOPMENT AND ANOXIA

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Activity-related changes in extracellular K^+ concentration and pH were studied in the adult and neonatal rat cortex, corpus callosum (CC) and spinal cord, concomitantly with the extracellular space (ECS) volume fraction (α) and tortuosity (λ) - the parameters affecting the diffusion of substances in CNS. Concentration-time profiles of TMA^+ applied by iontophoresis were quantitatively analyzed. The ECS volume in the adult rat cortex, CC, spinal cord and cortical slices occupies about 20 % of the total tissue volume (α = 0.20, λ = 1.6). At P4-6 the ECS volume was almost double that of adult rats, α = 0.37-0.40. At P12 the α gradually decreased to about 0.22-0.24 in gray matter and at P21 to about 0.18 in white matter. In spinal cord of adult rats electrical stimulation or peripheral injury evoked a shrinkage of the extracellular space by 20-50 % (Svoboda and Syková, Brain. Res. 1991), while no changes in α were found in P4-6. It is evident that the changes in ECS diffusion parameters are closely related to gliogenesis and to activity-related swelling of glia. Intrinsic and systemic properties of the extracellular microenvironment during and after ischemia were compared. As blood pressure fell, K^+ rose to 60-70 mM and pH fell by 0.6-0.8 pH units α decreased to 0.04-0.07 and λ increased to 2.00-2.20. At 10 and 30 min after this recovery α increased above "normoxic" values, to 0.25 and 0.30, respectively. *In vitro* α simply recovered from a reduced value during anoxia. The *in vitro* and *in vivo* differences during re-oxygenation point toward systemic mechanism of injury such as edema in this phase. Our results suggest that during and after ischemia the changes in the ECS diffusion parameters may aggravate or diminish accumulation of ions, neurotransmitters, metabolic and toxic products, and thus contribute to or prevent nervous tissue damage.

ON LINE MONITORING OF CELL VOLUME AND OSMOLYTE CONCENTRATIONS IN GLIAL CELLS AFTER OSMOTIC STRESS BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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Many pathological processes in the brain are accompanied with cell swelling of glia cells. Multinuclear NMR spectroscopy is a new biochemical tool to monitor non-invasively the volume increase or decrease as well as variation of intracellular concentrations of various organic osmolytes. Diffusion weighted proton NMR indicates a 65% volume increase (within 3 min. i.e. minimal recording time) under hypotonic conditions (180 mOsm) or decreases by 40% in the presence of hypertonic conditiones (440 mOsm).

The energy charge (from ^{31}P NMR) remains constant, wheras the intracellular pH drops by 0.1 units during hypotonic stress or increases by 0.08 units during hypertonic stress. Both are regulated within 2 hours to normal values.

Several organic osmolytes vary their concentrations in the short term and long term osmotic stress. Glycerophosphocholine and glycerophosphoethanolamine drop below NMR detectability within 1 hour and are resynthesized within 1 hour after restoration of isotonic situation. In the long term hypotonic stress (24 h) C6 glioma cells loose more than two thirds of their myo-inositol (ins) (an astroglia specific marker in the NMR) and taurine (tau). Similar decreases of the steady state concentration of glycine (gly), glutamine (gln), alanine (ala) and other amino acids to a minor extend are observed. Hyperosmotic stress provokes only moderate increases of myo-ins and tau, but much stronger increase of gln and gly.

Different primary glia cells have different steady state concentrations of organic osmolytes i.e. astroglia show predominantly myo-ins and tau, wheras oligodendrocytes reveal also tau but no myo-ins. In this case oligodendrocytes are more similar to neurons than to astocytes.

MULTIREGULATION OF THYROID HORMONES DEIODINASES IN ASTROGLIAL CELLS.

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In the central nervous system thyroid hormone metabolism is precisely controlled by several mechanisms to maintain brain T_3 content. In the brain, 3,5,3'-triiodothyronine (T_3) is locally produced by monodeiodination of thyroxine (T_4) catalyzed by a type II deiodinase (DII). T_4 and T_3 are degraded into inactive metabolites by a type III deiodinase (DIII). Astroglial cells in culture contain both enzyme activities. We show herein that DII and DIII activities are regulated by multiple pathways.

Factors activating protein kinase A (PKA), phorbol esters activating protein kinase C and acidic Fibroblast Growth Factor (aFGF) activating FGF-receptor tyrosine kinase, strongly induced DII activity (up to 60-fold the basal level). All these inducers increased the 30 kDa substrate binding subunit of DII, as demostrated by affinitty labeling. Moreover, T_4 and T_3 rapidly inhibited DII activity and TSH itself induced DII activity by a cyclic AMP independant pathway. Factors activating PKA (Bromo-cAMP, isoproterenol) were poor inducers of DIII activity while phorbol esters and aFGF strongly induced DIII activity (up to 120-fold the basal level). T_3 induced DIII activity and potentiated the effect of other factors (Bromo-cAMP, TPA, aFGF). Retinoic acid was a potent inducer of DIII activity. All the inductions of DII and DIII activities were always observed after several hours and probably require many steps including protein synthesis. Both the relative potencies of the inducers and the time-course of their actions on DII and DIII activities were different.

In conclusion, the multiregulation of astroglial deiodinases, which respectively catalyze T_3 production and T_3 degradation, probably plays a key-role in determining the cerebral availability of T_3 which controls brain development.

STUDY OF RADIALINE, AN ANTIGEN OF RADIAL GLIA EXPRESSED DURING NEURONAL MIGRATION.

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The main role of the radial glial cells in the developping nervous system is to serve as guide to the postmitotic neurons migrating from the periventricular zone to their final position in the neocortex. The interaction between this glial type and the neurons during their migration remains poorly understood. To characterize further the radial glia and understand its function, we have used a monoclonal antibody named RC2 which labels exclusively the radial glia population during the neuronal migration. Immunohistochemical labelling with RC2 of embryonic mouse neocortex results in an homogenous staining of radial glial cells from their cell bodies in the periventricular zone up to their end-feet in the subpial zone. Staining with RC2 has also been obtained in cultures of 2 day-old mice cerebellar microexplants from which long radial processes with terminal arborisation and end-feet can be seen. In Western blots, the RC2 antibody recognizes several antigens in the late embryonic mouse neocortex whose molecular weights are respectively 200, 165, 145 and 35 kDa. The two proteins of intermediate molecular size are the most abundant ones and have been further characterized, receiving the provisionnal name of Radialine. Both proteins seem to be located intracellularly and can be extracted only with ionic detergents such as SDS suggesting that they could be associated with the cytoskeletton. Their expression is exactly matched with the immunohistochemical staining and corresponds to the period during which neuronal migration occurs in the neocortex. The two proteins have been partially purified from SDS extracts of mouse neocortex by preparative electrophoresis. After this step, they can be maintained in solution in buffers containing 6 M urea. They are both N-glycosylated. The two Radialine proteins are now subjected to further purification using ion exchange chromatography in order to obtain preparations that could be used for sequencing.

TUMOR NECROSIS FACTOR ALPHA (TNF-α) IS INVOLVED IN THE PATHOGENESIS OF THE PANENCEPHALOPATHIC TYPE OF CREUTZFELDT-JAKOB DISEASE (CJD)

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CJD occasionally appears as the panencephalopathic type with severe involvement of the white matter. Intraocular injection of recombinant (r)TNF-α produces lesions in the optic nerve indistinguishable from those reported for this type of CJD. The lesions were patchy and confined to the injected optic nerve. Axons showed variable features of degeneration. Numerous vacuoles distended the myelin sheath. Hypertrophic astrocytes were numerous and many active macrophages containing digested myelin debris and lyre-like paracrystalline bodies. Thus, lesions produced by TNF-α mimic those of the panencephalopathic type of CJD. To substantiate further the hypothesis of TNF-α involvement in the myelin damage in CJD, we applied Western blot and RT-PCR to detect TNF-α and its mRNA in CJD-infected mouse brains. Our data indicate upregulation of both TNF-α and its mRNA. In conclusion, TNF-α seems to play in CJD the role analogous to that of other demyelinating conditions

TRANSIENT EXPRESSION OF TENASCIN AND CHONDROITIN SULFATE PROTEOGLYCANS FOLLOWING TRANSECTION OF THE POSTCOMMISSURAL FORNIX

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Transection of the adult rat postcommissural fornix induces spontaneous sprouting of fornix fibers through an astrogliotic milieu up to the lesion site, where the growing fibers stop (Wunderlich et al., 1994, Glia, in press; Stichel and Müller, 1994, this issue). Recent observations suggest that the barrier formed after CNS lesions reflects the expression of repulsive extracellular matrix molecules, such as tenascin (TEN) and chondroitin sulfate proteoglycans (CS-PG). In order to obtain further insight into the molecular composition of the barrier after fornix transection we analysed the post-lesion (PL) expression of the putative inhibitory factors TEN and CS-PG using monoclonal antibodies (TEN, Chemicon; CS56, Sigma; 3B3, ICN).

As early as 2 days (d) after transection we observed the appearance of TEN-immunopositive glial cells around the lesion center. The number of these cells increased, reached a peak at PL3-5d and disappeared until PL4 weeks (w). No TEN-positive cells were seen in the proximal or distal fornix tract. CS56- and 3B3-immunocytochemistry resulted in diffuse, extracellular staining patterns that appeared at PL3d immediately adjacent to the lesion center. While the staining intensities gradually decreased after 3d and 1w, respectively, the immunocytochemical stainings disappeared until PL5w and PL6 months.

Our study showed that TEN and CS-PG appeared early and transiently in a restricted area around the transection site of the adult rat postcommissural fornix. The distribution and timing of TEN and CS-PG expression suggest that both molecules could serve to define repulsive territories for regenerating axons.

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INFLUENCE OF TRANSPLANTED ASTROBLASTS ON THE LESION-INDUCED EXPRESSION OF TENASCIN AND CHONDROITIN SULFATE PROTEOGLYCAN

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Transplanted astroblasts significantly increase the number of sprouting fibers in the transected fornix tract (Wunderlich et al., 1994, Glia, in press). However, the regenerating fornix fibers do neither penetrate the graft nor cross the lesion site but stop on the surface of the transplant. While the growth-promoting effect of astroblasts might be explained by the down-regulation of diffusible growth-inhibitory factors (Lips et al., 1994, this issue), the formation of a barrier might indicate the local expression of these molecules. In this study we analyzed the influence of implanted astroblasts on the lesion-induced expression of tenascin (TEN) and chondroitin sulfate proteoglycans (CS-PG) using immunocytochemical methods (TEN, Chemicon; CS56, Sigma; 3B3, ICN). The injection of DMEM or saline served as control.

While the TEN staining disappeared after transplantation of either astroblasts, DMEM or saline, the CS56- and 3B3-stainings were specifically influenced by transplanted astroblasts. The latter cells increased the staining intensities and prolonged the expression CS-PG. Animals with astroblast-transplants exhibited CS56- and 3B3-immunoreactinity even after 18 months post-lesion at the transplant boundary.

Our study suggests that astrocyte-induced changes in the expression of TEN might be involved in the stimulation of spontaneous sprouting, while the distribution and timing of CS-PG expression after transplantion suggests a role for CS-PG in defining the transplant barrier.

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DEVELOPMENTAL PROFILES OF (SULFO)GALACTOLIPIDS PRODUCED IN VIVO AND IN VITRO BY OLIGODENDROCYTES

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(Sulfo)galactosyl ceramides, GalCer and SGalCer, are glycolipids that are typical for the myelin membrane[1]. Characteristic features of the ceramide backbone of these molecules are: (i) N-linked very long-chain fatty acids (VLCFA; predominantly 24:0 and 24:1); (ii) 20-70% of these acyl chains is hydroxylated at the 2-position (αHFA-species); (iii) a relatively high content of uneven acyl chains due to a process of α-oxidation. Physicochemical data indicate that (S)GalCer species containing VLCFA and αHFA contribute to the stability of compact myelin.

Normal and reverse phase HPLC techniques are used to separate perbenzoylated derivatives of the various species of (S)GalCer.

The specific aims of this study are to analyse the species composition of (S)GalCer as a function of development both in vivo and in vitro (primary culture of oligodendrocytes derived from rat spinal cord) in order to:

1. Correlate the (S)GalCer-species profile (chain length and % αHFA) with the maturation of the myelin membrane; specifically, compare the profile in vivo with that in vitro (without neuronal elements).
2. Identify factors regulating the enzymatic processes of α-hydroxylation and α-oxidation, which are but poorly understood.

A previous report[2] on the accumulation of (S)GalCer species in vivo in cerebra and spinal cord will be supplemented with data obtained with rat oligodendrocytes in primary culture.

[1]J.P.Vos, M.Lopes-Cardozo and B.M.Gadella (1994) Biochim. Biophys Acta (Review) in press: Metabolic and functional aspects of sulfogalactolipids.

[2]M.Lopes-Cardozo, J.P.Vos and C.G.M. de Haas (1993) J. Neurochem. 61, S222D: Molecular species of sulfatides and galactocerebrosides in developing rat brain and spinal cord.

REGULATION OF GLIAL FIBRILLARY ACIDIC PROTEIN EXPRESSION IN CELL TYPE OF NON-NEUROECTODERMAL ORIGIN

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Glial fibrillary acidic protein (GFAP), the major component of vertebrates astroglial intermediate filament, is also expressed in certain cell types outside the CNS. Each so far described extraneural expression of GFAP is specific of one tissue and one animal species.We are particularly interested in mouse lens epithelial cells (LEC) and hamster adrenocortical expression of GFAP.
We previously showed that among mouse species or hamster species, extra-neural GFAP expression revealed a polymorphism. Only certain species present extra-neural GFAP expression.
Here we show that DNA sequences directing transcription do not seem involved in permissivity of GFAP expression in extra-glial cells. So we investigate further in protein synthesis process.
We have used competitive polymerase chain reaction (PCR) technique to determine the levels of GFAP mRNA expression in mouse LEC and hamster adrenocortical cells from species that express or not the protein GFAP. The presence of GFAP mRNA was detected in LEC and adrenal gland of species that do not express the protein.
These studies confirm the expression of GFAP in non-neural tissues, and suggest that such ectopic expression may be controlled at a post-transcriptional level

SEQUENCE OF DEVELOPMENT OF OLIGODENDROGLIAL CELLS IN THE ENCEPHALON OF Gallotia-galloti. GOLGY TECHNIQUES STUDY.

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The oligodendroglial sequence of Gall-gall has been studied with Golgi-Hortega, Stensaas and Kospch techniques.
Based on morphological features, two types of cells have been observed giving rise to oligodendroglial cells: glioblast and radial glia through astroglial precursors. Glioblasts, which are observed from E-33 to adult specimens, are round cells with short processes. Oligodendroblasts, observed in hatching, show a citoplasm with soft processes which surround a big circular nucleus. These cells would be able to become the inmature oligodendroglial types that appear from E-39 to adult specimens, which are given to the next five mature types from E-38 to adult: velate type placed in grey matter and bundles, type I in grey matter mainly as vascular and neuronal satellite and in thin bundles as interfasciculars, type II in white matter detached fibers, type III in very thick bundles and type IV in the bundles, above all, in caudal zones.
Besides, from E-38 to adult, the mossy type has been described, whose origin has been stablished from radial glia.A similar sequence could be described in adult specimens where glioblast as well as radial glia stay. This study complements previous one using electron microscope and immunochemistry (Monzón-Mayor,'90,'91; Yanes,92) and confirms the stablished model in different species(Rio-Hortega,'28;Ramón-Moliner,'58;Puelles,'78).

Ramón-Moliner, 58, J. Comp. Neurol. 110, 2, 157-171.
Rio-Hortega,'28, Mem. Soc. Esp. Hist. Nat.,XIV.
Monzón-Mayor et al.,'90,'91,J. Anat. 170,43; 174,251.
Puelles et al.,'78, Neurosc. 3, 307-325.
Yanes et al.,'92, Anat. Embryol. 185,475-487.

DIFFERENTIATION OF TYPE-2 ASTROCYTES AND EXPRESSION OF GFAP AND K-OPIOID RECEPTORS IN RAT CEREBELLAR GRANULE CELL CULTURES UNDER THE EFFECT OF CHANGEING K^+ CONCENTRATION

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During the early ontogeny of the nervous system several signals control the survival and differentiation of the neuronal and glial cells. Recently the trophic effect of opioids acting through specific receptors was also demonstrated. Granule neurons of rat cerebella need an excitatory signal in a critical period of differentiation resulting an increased intracellular Ca^{2+} level.
Primary cultures from 8-day-old rat cerebella are mixed population of granule neurons and astrocytes. We find that the concentration of K^+ in the medium influences strongly the neuronal survival and parallel the glial growth and differentiation. Minimum two populations of glial cells are in the cultures, they correspond morphologically to astro-1 and astro-2. Both are $GFAP^+$ but astro-2 cells are also rich in kappa-opioid receptors as double immunocytochemistry by the application of the specific monoclonal antobody KA8 showed. The astro-2 glial differentiation was promoted while astro-1 glial growth was suppressed in relation to the neuronal survival. The signal could be the potassium directly, but also kappa-opioid agonists produced by granular neurons can be supposed as trophic factors for glial cells.

INDUCTION OF CYCLOOXYGENASE AND LIPOCORTIN-1 IN REACTIVE ASTROCYTES IN KAINATE-LESIONED CEREBELLUM

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Many studies have shown that cultured astrocytes are able to synthesise and release prostaglandins (PGs), and it has been suggested that astrocytes could constitute the major cellular compartment for the synthesis of these compounds *in vivo*. The key enzyme in the synthesis of PGs, cyclooxygenase, is known to exist in both constitutive and inducible forms. We have found that whereas normal astrocytes *in vivo* express neither form, reactive astrocytes in kainate-lesioned rat cerebellum express the inducible form of the enzyme. Similarly, astrocytes cultured in the presence of serum also express the inducible enzyme, whereas in the absence of serum the enzyme is downregulated. These data suggest that PG release from astrocytes *in vivo* is a consequence of damage rather than normal function. Another important molecule in the inflammatory cascade is the 35kDa. protein lipocortin-1. The exact fuction(s) of this protein is not clear, but several studies have implicated it in the suppression of inflammatory responses in many tissues. We have also shown that this too is upregulated in reactive astrocytes and is expressed by cultured astrocytes. Taken together these observations imply a role for reactive astrocytes in inflammatory conditions in the CNS.

CROSS BREEDING OF P0 KNOCK-OUT AND MBP-DEFICIENT (SHIVERER) MICE REVEALS DISTINCT, BUT ALSO PARTIALLY OVERLAPPING FUNCTIONS OF MYELIN GENES.

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Based on the observation that Schwann cells of P0-deficient mice form major dense lines in compacted and semi-compacted aspects of their abnormal myelin sheaths, the hypothesis was tested whether MBP could functionally replace P0 in the intracellular compaction of myelin. For this purpose, double mutants were generated which lack both P0 and MBP by cross breeding P0-deficient and shiverer mice. The myelin-like sheaths in peripheral nerves of such double mutants indeed did not form major dense lines and were totally uncompacted. Additionally, the corresponding axonal diameters were significantly reduced. Animals expressing half the complement of P0 and lacking MBP formed normally compacted myelin of strongly reduced thickness. Our results show that P0 can be partially replaced by MBP in myelin compaction and vice versa. Additionally, both molecules appear to be involved in the determination of myelin thickness.

GLIAL PROGENITOR CELL REPOPULATION OF IMMUNODEPLETED MIXED GLIAL CELL CULTURES.

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Glial cell cultures prepared from postnatal forebrain contain astrocytes, oligodendrocytes and glial progenitor cells with representatives at various stages of differentiation. In such cultures the potential of glial progenitor populations to generate mature glial phenotypes is considerable. This is evident following depletion of mature glial phenotypes from mixed glial cell cultures where there is replacement of glial cells by the progenitor population, however, the dynamics and control of this response are unclear. The present study was undertaken to identify the potential of pre-A2B5 progenitor phenotypes by unmasking their differentiation and proliferation in vitro following removal of more mature O-2A glial phenotypes from forebrain mixed glial cell cultures. Over a 12 day interval following immunodepletion of oligodendrocyte lineage cells, a 4-fold increase in the numbers of top-dwelling cells occurs. The majority of these new top-dwelling cells express A2B5 and in the initial phase of the response about 35% are proliferating (shown by BrdU incorporation). A progression from a proliferating A2B5-negative-, through a proliferating A2B5-positive-population to the emergence of a small population of mature non-dividing top-dwelling cells is suggested. The existence of proliferating A2B5-negative progenitors in these cultures and their potential for replacing oligodendrocyte lineage cells has implications for cell lineage and transplantation studies which require purified astrocyte and oligodendrocyte cultures.

INACTIVATION OF NEUROPEPTIDES BY GLIAL CELLS

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Since astrocytes embrace synapses in the CNS, they are strategically located to participate in the inactivation of interneuronal signal molecules. Their role in uptake and metabolism of amino acid and catecholamine transmitters is well established. In analogy, astrocytes might also be involved in the inactivation of neuropeptides. Cultivated rat cortical astrocytes hydrolysed rapidly neuropeptides of 8-15 amino acid chain length, including neurotensin, somatostatin, bradykinin, angiotensin II, substance P and LHRH, but not peptides of shorter or longer chain length (e. g. enkephalin, neuropeptide Y). By use of specific inhibitors and analysis of cleavage sites the metallo-endopeptidases EC 3.4.24.16 and EC 3.4.24.15 previously purified from total rat brain homogenate [1] were identified as the predominant astrocytic neuropeptidases. Rat microglia cells as a possible source of contamination in cultivated astrocytes rapidly hydrolysed enkephalin by action of aminopeptidase M (CD 13, EC 3.4.11.2) which could be immunostained on these cells and was upregulated in co-culture with astrocytes. Thus, glial cells must be considered to be an important site for the proteolytic inactivation of neuropeptides.

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PROSTANOID BIOSYNTHESIS IN ACTIVATED MICROGLIAL CELLS: REGULATION BY THE β-ADRENERGIC AGONIST ISOPROTERENOL AND INTERFERON-γ

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The presence of prostanoids such as prostaglandins (PGs) and thromboxanes (TXs) in the central nervous system (CNS), has been known for some time. These arachidonic acid (AA) metabolites are believed to be important regulators of local immune and inflammatory processes, but the mechanisms triggering their synthesis and release in the CNS are still poorly understood. In addition to astrocytes, microglial cells, belonging to the macrophage-monocyte lineage, represent an important source of these active compounds. In order to better understand AA metabolism and its regulation, we have undertaken studies on cultured rat microglial cells, activated by exposure to bacterial lipopolysaccharide (LPS). Microglia released PGE_2, PGD_2 and TXB_2 in response to LPS in a dose-dependent fashion (1-100 ng/ml). A lag-phase of 4 - 6 hours was observed before significant amounts of prostanoids could be detected in the culture media. LPS was also able to increase the release of ^{3}H-AA from pre-labelled microglial cells. The ability of the protein synthesis inhibitor cyclohexymide to prevent both LPS-elicited biosynthesis of prostanoids and AA release, together with the delayed production, suggest that LPS induced rather than activated phospholipase A_2, the enzyme releasing AA from membrane phospholipids. In addition, experiments performed in the presence of exogenous AA, confirmed that the supply of AA is rate limiting for prostanoid biosynthesis. Isoproterenol, a β-adrenergic agonist, did not affect prostanoid biosynthesis, when administered alone or in combination with exogenous AA; however, it synergized with LPS, causing approximately a two fold increase in PGE_2, PGD_2 and TXB_2 production. On the contrary, the inflammatory cytokine interferon-γ inhibited the release of prostanoids stimulated by LPS and also counteracted the potentiating effect of isoproterenol.

EFFECT Ca^{2+} AND TEMPERATURE ON ^{3}H-OUABAIN AND ^{3}H- BUNGAROTOXIN SPECIFIC BINDING OF THE GLIOMA CELLS.

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The role of Ca^{2+}-dependant membrane fluidity in regulation series of Na, K-ATPase and Acetylcholine receptor of glioma cells has been investigated. It was found that ^{3}H- ouabain (10^{-7} M) and ^{3}H-bungarotoxin (10^{-8} M) bound cells depend on extracellular Ca^{2+}-concentration and temperature.From 0^{o}C to 45^{o}C ^{3}H-ouabain specific binding decreased when Ca^{2+}-concentration increased and had maximal level at 22^{o}C. ^{3}H-bungarotoxin binding of membrane of glial cells was risen when extracellular Ca^{2+} changed from 11 to 55 mM. These dates was interpreted as a possible transient of fluidity of glial membrane lipids during Ca^{2+} and temperature effects and regulation member of sites of Na,K-ATPase and acetylcholine receptors.

ULTRASTRUCTURAL CHANGES DURING PROCESS FORMATION STUDIED IN A *LOW DENSITY* MODEL OF SUBCULTURED ASTROCYTES

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Some characteristic shape features of astrocytes can be induced to develop under in vitro conditions, e.g., radially oriented processes. Depending on medium conditions and time in vitro, however, astrocytic shape may vary between epitheloid and stellate form. Astrocytes have the capability to change their morphology according to local conditions. In order to relate ultrastructural changes to individual cells, we have established a very low density model of subcultured astrocytes (plating density <800/mm^2). Subcultured (type 1) astrocytes *in vitro* are known to grow in a polygonal, flat shape. We have used cAMP analogues such as dibutyryl (dB) cAMP to induce shape changes into multipolar cells predominantly caused by retraction of cytoplasm. In contrast to previous studies focussing on alterations of the cytoskeleton, we were interested in the early reactions (15 min to <4 h) of the membrane dynamics to dBcAMP application, and mechanisms involved in the retraction process. Preliminary results indicate various types of increase in cell surface area including skeletonized ("mossy-like") endoplasmic reticulum. There are abandoned primary and secondary lysosomes. In contrast, vesiculation in control cultures is confined to small subsurface vacuoles lining the cell membrane. In addition, the *morphology* of subcultured astrocytes of the polygonal, flat type (type 1) could be influenced towards the stellate shape by growing cells in a conditioned medium (CM) of high density astroglial cultures from DIV1 onwards. Under CM process formation is initially and proceeds through growth cone-like knobs containing large vacuoles rather than through retraction of cytoplasm.

GLIAL FIBRILLARY ACIDIC PROTEIN, VIMENTIN AND S100 PROTEIN IN POSTNATAL MONKEY VISUAL CORTEX: TRANSIENT REDUCTION OF GFAP-IMMUNOREACTIVITY

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In the primary visual cortex of *Callithrix jacchus*, an animal model chosen for its distinct developmental biology determined by a general developmental delay *in utero* and a rapid behavioural development postnatally, we studied the postnatal maturation of astrocytes using antibodies against GFAP, vimentin and S100 protein as immunohistochemical markers. For semi-quantificative evaluation of immunoreactivity (IR) image analysis of staining intensities was adopted. In the cortical grey matter of this species, GFAP-IR is high at birth (130% of the adult value), decreases until about 3 months of age (80%) and increases again towards adult values (100%). Vimentin expression was also high at birth with IR mostly being confined to radial glia fibers. Thereafter, vimentin-IR showed a decline towards 3 months and later. S100 staining of neuropil, in contrast, augmented postnatally and revealed a laminar shift of maximal IR from layer IV to supragranular layers during ontogenesis. The decrease of GFAP-IR is predominantly due to changes in the numerical density of GFAP+ astrocytes within cortical tissue (newborn: 18,600 GFAP+ astrocytes/mm^3; 1 month: 11,600/mm^3; 3 months: 5,700/mm^3; 7 months: 9,700/mm^3; adult: 10,200/mm^3), while the overall number of astrocytes apparently remained more or less constant as shown by S100-IR of astrocytic somata. At the time of low GFAP- IR a reduced area density of intermediate filaments was found in astrocytes by electron microscopy. The time period of reduced GFAP-expression coincides with a period of prominent synapse remodeling and elimination in the visual cortex of marmoset monkeys.

MET-ENKEPHALINERGIC SYSTEM AND GLIOMAS

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Endogenous opiate peptides and more particularly Met-enkephalin, by inhibiting cell replication, play a role in the growth regulation of normal cells, especially astrocytes, and different tumoral cell types.
We address whether the Met-enkephalinergic system (peptide and receptors) assume such a regulatory function during astrocyte transformation.
The results gained show that :
- *in vivo* as well as *in vitro*, human gliomas express the proenkephalin gene and synthetize Met-enkephalin. However, these activities decrease with increasing malignancy.
- cultured human glioma cells are unresponsive to exogenously applied Met-enkephalin. The addition of this peptide into the culture medium, even at high concentration (10 uM) do not result in an inhibiton of growth.
- these cell lines are devoid of Met-enkephalin binding sites. Tumoral tissues from surgical specimens do possess such receptors but the percentage of gliomas expressing Met-enkephalin receptors decrease with increasing malignancy.
These modifications (ie, down regulation of Met-enkephalin and receptor expression and absence of response to Met-enkephalin) suggest that alterations in the Met-enkephalinergic system may take place during malignant transformation of astrocyte and thus play a role in tumoral progression.

SWELLING-INDUCED CHANGES IN TRANSMEMBRANE POTASSIUM FLUXES IN C6 GLIOMA CELLS

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Hypotonic swelling of C6 glioma cells, placed into the medium with osmolality 180 mOsm, stimulated 86Rb+ (K+) efflux by 2.8-3.5 times. The volume-sensitive efflux of radiotracer was blocked by 45% and 41% with 0.5 mM quinidine and 1 mM BaCl2, respectively, but was insensitive to 0.5 mM furosemide and 10-50 μM bumetanide. These data show that swelling activated several types of K+ channels but not electroneutral K+/Cl- or Na+/K+/2Cl- symport in such cells. At the same conditions the inward-directed 86Rb+ (K+) flux was stimulated 1.8-2.0 fold, being mediated by increase in ouabain-sensitive (sodium pump) and bumetanide-sensitive (Na+/K+/2Cl- cotransport) components of the isotopic influx. The sodium pump and Na+/K+/2Cl- cotransport were activated by 30-60% and 180-220%, respectively. The possible role of activation of K+ influx for the replenishing the cytoplasmic loss of this cation in the process of regulatory volume decrease (RVD) and in post-RVD period in the glial cells is discussed.
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SWELLING-INDUCED POTASSIUM UPTAKE IN CULTURED PRIMARY RAT ASTROCYTES

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When rat brain primary astrocytes were placed into the medium with osmolality 180 mOsm the 86Rb+ (K+) efflux was stimulated 3.8-4.6-fold. 86Rb+ (K+) influx was increased 1.6-1.8-fold under the same conditions. The outward-directed net flux of K+ underlied a volume recovery of astrocytes in hypotonic medium as described by Kimelberg and Goderie (In: Norenberg, Schousboe and Hertz (Eds) Biochemical Pathology of Astrocytes, Alan R. Liss, NY, 1988, pp. 299-311) and was mediated by activation of potassium channels (Kimelberg and Kettenmann, Brain Res., 1990, 529, 255-261; Sanchez-Olea et al., Am. J. Physiol., 1993, 264, C836-C842). Using 1 mM ouabain and 10 μM bumetanide it was shown that the stimulation of influx was caused by increase in the activities of sodium pump and Na+/K+/2Cl- cotransport by 70-100% and 30-50% relative to a basal level, respectively. The possible physiological role of activation of K+ influx under regulatory volume decrease is discussed.

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MICROGLIA, AN EARLY MARKER OF NEUROTOXICITY.

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In the search for early markers of neurotoxicity, the responses of microglial cells and neurons to chronic treatments with different metal compounds were analyzed *in vitro*, using aggregating brain cell cultures of fetal rat telencephalon as a model. Differentiating cultures were treated during 10 days with different concentrations (ranging from 10^{-10} to 10^{-4} M) of lead acetate, mercury chloride, dimethylmercury and trimethyltin. Microglia were found to be the most sensitive cell type, since an increased number and clustering of *Griffonia simplicifolia*-positive cells could be observed at the lowest concentrations of the different metal compounds (10^{-10}–10^{-8} M of trimethyltin; 10^{-9}-10^{-6} M of mercury chloride and 10^{-8}-10^{-6} M of lead acetate and of dimethylmercury). When the concentrations increased, no change in microglial cells was found. The neurons, in contrast, appeared to be less sensitive, since a decrease in neuron-specific enzyme activities was observed only at the highest concentrations tested (10^{-6} M of trimethyltin and mercury chloride, and 10^{-4} M of lead acetate), and no effect was found after treatment with dimethylmercury. These results show that microglial cells respond to a toxic insult before any neuronal change could be detected. Microglia may be the first target of the metal compounds and their reaction may trigger the responses of other cell types. On the other hand, it cannot be excluded that discrete neuronal changes could act as an activating signal for microglial cells.

ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL STUDIES OF THE ASTROCYTES DURING THE ONTOGENY OF THE Gallotia galloti (REPTIL LACERTIDAE).

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Astrocyte development was investigated with E.M. and immunohistochemically (Vim-GFAP-GS) in the midbrain of the lizard.
With E.M. immature glioblasts were present until hatching, dark and early glioblasts could be identified from E34 and radial glia from E35 until adulthood. Astroblasts appeared at E35 with a characteristic rough endoplasmic reticulum.
The quantity of gliofilaments increased with the age and, in the adult, grey matter had very large amounts. Vimentin immunoreactivity appeared first in the ventricular walls in radial fibers and subpial end-feet at embryonic stage 32, it increased between E34/35 and decreased after 35 until the adulthood. GFA-P immunoreactivity gradually appeared in the same structures between E34 and E37 and increased until adulthood. Thus, in developing Gallotia galloti midbrain a shift from Vimentin-containing to GFAP-containing intermediate filaments begins around E36 or E37. Immature astrocytes appeared with the same shift at E40. GFAP-positive radial glia coexist with astrocytes in adult lizard. Optic tectum, Tegmentum and Isthmic nuclei are richest in GFAP-positive radial fibers. Glutamine synthetase immunoreactivity appeared at E35 in radial processes, increased until hatching and decreased in adult but it did not disappear. The scattered star and pear-shaped cell bodies became more immunoreactive with perivascular end feet. At E40 even in the adult, we observe hight positive Vim, GFAP and GS cells apparently "migrating" out the ventricular zone, normally from the "sulcus".
The appeareance of the endoplasmic reticulum with floculent material is a feature observed at E35 with EM and GS, that verify the nature of this marker in cells that begin to differentiate.
This work proves that the proliferation and maturation of astrocytes occur in the lizards in the last embryonary stages while in mammals they are postnatal (Dalh, 85). The antibodies used are phylogenetically stable. The GFAP astrocytes are present in lizard mesencephalon and they are absent in telencephalon (Yanes et al 90). We observe radial glia and matricial zones or "sulcus" even in adulthood. These facts could be to indicate the procedure of astroglial cells from radial glia or from "proliferative zones".

GENE ACTIVATION FOR NEUROTROPHIC FACTORS IN HUMAN GLIAL CELL CULTURES

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Various cytokines, including the family of Neurotrophic Factors (NTFs), influence neuronal development and survival. Controversy exists on the time of production, the functions and the cellular sources of NTFs in the central nervous system. We investigated the expression of mRNA for NTFs in highly enriched populations of human astrocyte cultures. Cells were isolated from fetal brains and from adult temporal lobe. Total mRNA was extracted from cell pellets and reverse transcribed into cDNA; cDNA was subjected to PCR amplification using primers specific for several NTFs and the PCR fragments were separated through acrylamide gel electrophoresis. Results showed that human fetal and adult astrocytes constitutively express mRNA for NGF, BDNF, NT-3 and for TGF-β1; no detectable mRNA for CNTF was observed in our cultures. Of particular interest is the observation that adult astrocytes retain the capacity to produce NTFs. Any impaired synthesis and/or release of these agents could be of special relevance in adult neuronal degeneration.

POTASSIUM CHANNELS IN LEECH GLIAL CELLS

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Neuronal activity and synaptic currents may cause an increase of the extracellular K^+ concentration. Glial K^+ buffering is the most dominant process in the regulation of the extracellular K^+ concentration. Therefore, glial membranes possess a high potassium permeability, mediated by the activation of a large number of different K^+ channels.
We investigated single K^+ channels in exposed leech glial cells by using the patch-clamp technique. We characterized a 83 pS K^+ channel with a permeability ratio $P_K/P_{Na} > 19$, which is activated by excision of the patch. During bath-perfusion with physiological saline solution, flickering events could be observed at membrane potentials more negative than -60 mV. In symmetrical 96 mM KCl solution the current-voltage relationship showed inward rectification. Since the 96 mM KCl solution did not contain Mg^{2+}, an intracellular Mg^{2+} block could be excluded. The channel activation following patch excision could indicate Ca^{2+}- and/or ATP-dependence. Application of 1 mM ATP at the cytoplasmic face of the membrane did not change the single channel amplitude or the gating behaviour. The single channel current was reduced considerably by the application of 10 mM TEA or 10 mM 4-AP at the cytoplasmic face of the membrane, indicating very fast blocking kinetics of both inhibitors. The inward rectification and the assumed Ca^{2+}-dependence suggest an involvement in K^+ buffering.
In addition a 64 pS K^+ channel with a permeability ratio $P_K/P_{Na} > 28$ was characterized. Single channel events could be observed in the cell-attached and in the inside-out configuration. In the cell-attached configuration a reversal potential of 55 mV was determined. In symmetrical 96 mM KCl solution the current-voltage relationship showed moderate outward rectification. The single channel amplitude was decreased by 10 mM TEA applied at the cytoplasmic face of the membrane. Since the 64 pS K^+ channel is active in the cell-attached configuration, it may contribute to the maintenance of the membrane potential.

CALCIUM INFLUX THROUGH KAINATE RECEPTORS BLOCKS GAP JUNCTIONS ON BERGMANN GLIA CELLS IN CEREBELLAR SLICES

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We studied the characteristics of electrical coupling between Bergmann glial cells in mouse cerebellar slices of postnatal day 6 and 20. A combination of Lucifer Yellow injection and patch clamping cell pairs revealed that Bergmann glia of the immature cerebellum were not coupled. However, at day 20, dye and electrical coupling was abundant and could always be detected between cell pairs separated by a distance of up to 50 µm. The junctional conductance was voltage and time independent and could be blocked (to 25 % of control) by halothane. Alkalinization of cells (by applying NH_4^+) increased the junctional conductance by 50 % while acidification of the cell interior (by removing NH_4^+) decreased the junctional conductance by 30 %. The junctional conductance was blocked to 30 % of the control after activation of kainate receptors. This link is most likely mediated by the influx of Ca^{2+} via the receptor since the effect was not observed in Ca^{2+} free medium indicating that Ca^{2+} entry via the kainate receptor pore closed the gap junctions. The gap junction of Bergmann glial cells predominantly contains connexin 43 as revealed by combining the patch clamp technique and single cell PCR. These studies indicate that electrical coupling between Bergmann glial cells is developmentally regulated and is controlled by a variety of physiological stimuli.

IDENTIFICATION OF A FATTY ACID BINDING PROTEIN IN DEVELOPING CNS: FROM GENE TO PROTEIN.

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Fatty acid binding proteins (FABPs) constitute a multigene family with tissue- and differentiation-specific expression patterns. For a subfamily, the cellular retinoid binding proteins (CRtBPs), evidence has been provided that they are regulating the metabolism of retinoids. A member of the FABP family, mammary derived growth inhibitor/heart-FABP (MDGI/H-FABP), has previously been shown to act as an endogenous growth inhibitor and differentiation factor of mammary epithelium.

Using a PCR approach designed to identify MDGI-related genes, we have isolated a gene encoding brain-FABP (B-FABP), cloned its cDNA and expressed the protein in bacteria to generate antibodies. The promoter region of the gene contains several potential binding sites for POU-domain transcription factors. Northern analysis, *in situ* hybridization and immunohistochemistry of adult mouse tissues identified gene expression only in neural tissue, in particular in radial glia (Bergman glia), in satellite cells of various ganglia and in olfactory nerve ensheathing cells. The gene is transiently expressed in developing neuroepithelium in all brain ventricles and spinal cord starting from day 10 of gestation. An immunohistochemical analysis of regional expression patterns is in progress.

The specific expression of B-FABP in regions of developing neuroepithelium suggests an essential function in neuronal differentiation or migration.

FUNCTIONAL RECOVERY IN THE AUDITORY PATHWAY OF THE RAT FOLLOWING GLIAL CELL DEATH SUBSEQUENT TO ADMINISTRATION OF 1, 3 DINITROBENZENE.

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Previous studies within this unit have established that 30mg/kg of 1,3 dinitrobenzene (DNB) given in three divided doses over 24 hours results in damage to and death of glial cells within the brainstem of the rat. In particular, lesions occur within the auditory pathway and the severity of lesioning in the auditory pathway has been shown to dependent on the functional/metabolic activity within the pathway. Glial cell death is also accompanied by a deficit in physiological function. In the auditory pathway this can be measured as changes in the amplitude and latency of specific waveform components in the brainstem auditory evoked response (BSAER). In this preliminary study, recovery of the BSAER was measured for 8 days after administration of DNB in 10 rats. In the first two days following DNB administration, the amplitude of the BSAER components fell by between 50-100% of control values. Over days 3-8 there appeared to be oscillations in the recovery of the amplitude of the BSAER waveforms. Individual analysis of the BSAER in each rat showed that whilst there was considerable temporal variation in the recovery profile in each animal, there was still a general pattern in the recovery. In six animals, amplitudes of some the BSAER waveforms overshot control values. In one animal, the maximal relative increase in the amplitude of one waveform was 300%. The latencies of measurable waveforms over the recovery period, showed no significant increase with respect to control values. From this preliminary study we conclude that these the recovery of the BSAER reflects changes in the homeostatic processes governing recovery of the electrophysiological response. This may in turn reflect the functional status of the regenerating or recovering glial cells supporting the activity of neurones in the auditory pathway.

GLUTAMATE-AGONIST INDUCED Ca^{2+}-INFLUX IN VOLTAGE-CLAMPED LEECH GLIAL CELLS

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Neuropile glial cells of the central nervous system of the leech *Hirudo medicinalis* are depolarized by the glutamate-agonist kainate. We recorded membrane current, I_m, with the two-microelectrode voltage-clamp technique, and simultaneously monitored intracellular calcium, Ca^{2+}_i, using the fluorescence ratio signal of intracellularly injected fura-2, of neuropile glial cells exposed by removing the outer ganglionic capsule and overlying neuronal cell bodies (1).

Bath-application of kainate (5-20μM) caused large and often biphasic inward currents of 20-60 nA in glial cells voltage-clamped at their resting potential (-65 to -75 mV). Kainate appears to open non-selective cation channels in the membrane of neuropile glial cells (2). Inward currents caused by kainate were accompanied by an increase of the intracellular Ca^{2+}-concentration by 20-80 nM from a resting concentration of around 50 nM. In most cells repetitive application of kainate led to a gradual increase in both inward current and Ca^{2+}_i-rises. Removing extracellular Ca^{2+} in the presence of 0.5 mM EGTA completely abolished kainate-evoked Ca^{2+}_i-transients and reduced the inward currents. In the presence of the calcium channel blocker Ni^{2+} (2 mM) kainate-induced inward currents and Ca^{2+}_i-rises were reversibly suppressed. Since voltage-gated Ca^{2+} channels could not be opened by kainate in voltage-clamped glial cells, it is concluded that the intracellular Ca^{2+}-transients are due to Ca^{2+}-influx through cation channels gated by kainate. There was no entry of Ni^{2+} into the cells which could quench fura-2 fluorescence. This suggests a blocking action of extracellular Ni^{2+} on kainate-gated cation channels.

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GLUTAMATE STIMULATES AP-1 DNA BINDING IN RADIAL GLIA

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The effect of L-glutamate and its structural analogs on the expression of the family of AP-1 transcription factors was examined in cultured chick radial glia, namely, Bergmann and Muller glia cells. Glutamate, AMPA and Kainate evoke a dose-dependent increase in AP-1 DNA Binding activity as assayed by Electrophoretic Mobility Shift Assays. These response was blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) indicating that it is mediated by an AMPA/low affinity kainate receptor. Since both glial cell types surround glutamatergic synapses, they have ben considered as mediators of the modulation of the efficacy of these synapses, the present findings suggest that a long term modulation of the excitatory synapses by the glial receptors takes place at the level of a stimulus-transcription coupling of genes involved in such a regulatory process.

IDENTIFICATION OF NEURONAL MEMBRANE-ASSOCIATED BINDING PROTEIN AGAINST PLASMINOGEN THAT IS ONE OF THE MICROGLIA-SECRETORY PRODUCTS

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Microglia secrete several kinds of proteases extracellularly. As described previously, we identified three kinds of proteases, elastase, urokinase type plasminogen activator (uPA) and plasminogen (PGn) in microglial conditioned medium. To investigate the role of these proteases in the interaction with neurons, these proteases were added to the cultured neurons. Among these proteases, only PGn showed neurotrophic effects, such as stimulation of neurite outgrowth and the promotion of dopamine uptake. Since these neurotrophic effects of PGn have not been described, the action mechanism of PGn on cultured neurons was investigated. PGn was found to specifically bind to cultured neurons with two different dissociate constants (kD), 12 nM and 125 nM. Furthermore, ligand blott analysis showed that PGn mainly binds to a protein with molecular weight of 45 kDa, which is associated with neuronal plasma membrane. The protein was purified and analyzed for the amino acid sequence. The result showed that the 45 kDa PGn binding protein is rat α-enolase. This result was also confirmed by immunoreactivity of anti-rat α-enolase antibody. These results suggest that α-enolase on the neuronal plasma membrane is one of the target of microglia-secretory PGn and that the neurotrophic effects of PGn are elicited through the binding to the PGn binding protein.

GLIAL SECRETORY ACTIVITY ACCOMPANIES THE DEVELOPMENT OF THE VERTEBRATE NERVOUS SYSTEM

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In the vertebrate nervous system glial cells are demonstrable, which release a secretory material into the cerebrospinal fluid and in the extracellular matrix.

Immunohistochemical studies with an antiserum against this secretory material indicate that during ontogenetic development a secretory radial glial cell type can be labeled. This immunoreactivity is correlated obviously with differentiation processes of brain structures.

Experimental studies demonstrated that the removal of secretory glial cells in early chick embryos can induce on alterated pattern of brain differentiation.

The secretory product of these glial cells is characterized as a mixture of poorly soluble, self aggregating protein. At least one component of this protein mixture is glycosylated.

The early expression of the glial glycoprotein in ontogenesis, its release into the extracellular matrix and preliminary studies functionally directed experiments speak for a basic function connected with the development of nervous system.

SIMULTANEOUS MEASUREMENT OF INTRACELLULAR pH WITH MICROELECTRODES AND BCECF IN LEECH GIANT GLIAL CELLS

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The regulation of intracellular pH, pH_i, of giant glial cells in segmental ganglia of the medicinal leech is due to an electrogenic Na^+-HCO_3^- cotransporter, and a Na^+/H^+ exchanger in the absence of CO_2/HCO_3^- (1). We used this well studied, large cell to compare two different techniques to measure pH_i.

We have monitored the pH_i in this glial cell using the ratio of the fluorescent dye 2',7'-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF) injected iontophoretically into the cell, which provides pH_i measurements integrated over the whole, dye-filled cell, and double-barrelled, neutral-carrier pH-sensitive microelectrodes, which give local cytoplasmic pH_i readings. Changes of pH_i were recorded simultaneously using these methods in different buffer systems and at various external pH. In CO_2/HCO_3^--containing saline pH_i became more alkaline, and followed the external pH much faster and with larger amplitudes than in CO_2/HCO_3^--free saline. We found that the BCECF ratio signal and the pH-microelectrode reading had a similar course. It was attempted to calibrate the BCECF ratio signal according to the pH-microelectrode reading, and after introduction of nigericin, which H^+-permeabilizes the cell membrane, to obtain comparable values for pH_i.

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TTX TREATMENT OF ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES INFLUENCES IFN-γ INDUCED MHC CLASS II EXPRESSION, BUT NOT CONSTITUTIVE MHC CLASS I EXPRESSION OF ASTROCYTES

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Expression of MHC products in the intact CNS on glial cells is low or undetectable. However, under pathologic conditions like inflammation, injury or neuronal degeneration some glial cells express MHC products.

We are studying the regulation of MHC products on astrocytes in organotypic hippocampal slice cultures of neonatal Lewis rats by immunostaining of frozen sections. The level of expression is quantitated using a fluorescence microscope equipped with a video camera and image analysis software.

After one week in culture the hippocampal slices exhibited good neuronal differentiation. In these cultures the expression of MHC class II products on astrocytes was inversely correlated with neuronal outgrowth. In particular astrocytes in neuron rich areas were not induced by 100 U/ml IFN-γ to express MHC class II products and had only low levels of constitutive MHC class I products. Treatment of the explant with the sodium channel blocker TTX restored inducibility of MHC class II expression on astrocytes in neuron rich areas, but had no effect on constitutive MHC class I expression.

These results indicate, that active neurons inhibit induction of MHC class II products on astrocytes and that the inhibition can be abolished by blocking of neuronal activity with TTX.

GLIAL CELLS SYNTHETIZE AND RESPOND TO 1,25-DIHYDROXYVITAMIN D3

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The secosteroid 1,25-$(OH)_2D_3$ is the ligand of the vitamin D nuclear receptor (VDR), whose structure is closely related to that of the thyroid hormone or retinoic acid receptors. 1,25-$(OH)_2D_3$ was shown to increase the synthesis of nerve growth factor (NGF) in primary cultures of rat astrocytes. This response is accompanied by an induction of the VDR gene, and that of a gene encoding a vitamin D metabolizing enzyme, the 24-hydroxylase gene. Thus, besides the production of a neurotrophic factor, the hormone controls its own responsiveness and degradation in astrocytes. Conversely, microglial cells cultured in vitro, and activated with lipopolysaccharide or interferon g become able to convert the 25-$(OH)D_3$ precursor into the active metabolite, 1,25-$(OH)_2D_3$. This raises the possibility that in addition to a transit of 1,25-$(OH)_2D_3$ accross the blood-brain barrier, an intracerebral synthesis of the hormone may take place, possibly during inflammation. These data support the concept that 1,25-$(OH)_2D_3$ is a modulator of astrocytic plasticity. The hormone, which has a potent immunomodulatory role on peripheral lymphocytes or monocytes / macrophages, could also contribute to confer to the brain its special immunological status.

Identification of new cell-surface receptors on oligodendrocyte precursor cells

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Oligodendrocytes are the myelin-forming cells of the central nervous system (CNS). Cell-surface receptors regulating the behaviour of the precursor cells, in particular their interaction with neurones, are largely unknown. To identify and characterize new cell-surface molecules monoclonal antibodies were used as a tool. For the production of monoclonal antibodies rats were immunized with a mouse oligodendroglial precursor cell line derived by infection of primary cultures with a t-neu expressing retrovirus. This cell line interacts with axons *in vitro* and after transplantation into demyelinated lesions and therefore expresses cell surface receptors regulating the interaction of oligodendrocyte precursor cells and neurones. Either whole cells or glycoprotein fractions obtained via lectin affinity chromatography have been used as immunogens. A range of antibodies were obtained by this approach which are specific for surface antigens of oligodendroglial lineage cells. Several of these antibodies react in Western Blot and can be used to immunoprecipitate the protein recognised. These proteins are now under extensive study.

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Degeneration and Regeneration in the Hippocampus of Epileptic Rats: Tenascin Expression by Proliferating Astrocytes

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Temporal lobe epilepsy is associated with hippocampal lesion and sprouting of mossy fiber collaterals (i.e. the axons of granule cells which will make new synaptic contacts with granule cell dendrites within the dentate molecular layer).

In the present study by combining autoradiographic analysis of ^{3}H incorporation with immunocytochemistry, we demonstrated that in the fields showing a neuronal degeneration the glial reaction consisted in a proliferation and hypertrophy of astrocytes and microglial-macrophages. In contrast, in the fields showing an exclusive terminal axonal degeneration (i.e. the molecular layer), glial reaction consisted in an exclusive astroglial hypertrophy (neither glial cells proliferated nor microglial cells were activated). These results also demonstrated that oligodendrocytes did not proliferate in the hippocampus of kainate-treated rats.

We also shown that tenascin immunoreactivity, a member of the L2/HNK-1 family of adhesion molecules, was increased in the hippocampus of kainate-treated rats. This increase was particularly striking in the CA3 field, where the antibody mainly labelled proliferative (^{3}H-thymidine labelled) astrocytes. In contrast tenascin immunoreactivity did not change in the molecular layer. In vitro studies suggested that tenascin has repulsive properties and may constitute an unfavorable substrate for neurite outgrowth. Interestingly, in the present study the expression of tenascin immunoreactivity was observed within the zones without axonal regeneration (CA3) whereas the zones depicting a great pattern of sprouting were devoid of tenascin immunoreactivity (the molecular layer). We infer from the present results that tenascin may in situ impede the terminal sprouting of mossy fibers in CA3 and explain why mossy fibers do not innervate CA1 pyramidal cells in kainate-treated rats.

DEVELOPMENTAL CHANGES IN THE NUMBER, SIZE AND ORIENTATION OF GFAP-POSITIVE CELLS IN THE CA1 REGION OF RAT HIPPOCAMPUS

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The postnatal development of astrocytes was studied in the CA1 region of rat hippocampus in four age groups using a polyclonal antibody against glial fibrillary acidic protein (GFAP). The main proliferation of GFAP-positive cells (GFAPpc) occurs in all laminae between postnatal days 8 and 16. The number of GFAPpc per unit area is reached in stratum lacunosum-moleculare and stratum oriens at about two weeks and in stratum radiatum at about three weeks of age. During further development - at the age of 24 days - the orientation of individual GFAP-positive astrocytes in stratum radiatum becomes polar with an orientation almost perpendicular to stratum pyramidale. This was revealed by an analysis based on determination of the quotients between the angular orientation of the processes of single individual GFAP-positive cells. When the crossing points of all glial processes over vertical and horizontal grid lines were determined and respective quotients evaluated the same development towards a perpendicular orientation of astrocytes was noted in stratum radiatum. The same approach revealed a transient orientation parallel to the fissure in stratum lacunosum-moleculare around day 24. Camera lucida drawings of GFAPpc in stratum radiatum reveal that GFAP-positive astrocytes become larger during the first three postnatal weeks, followed by a reduction of various parameters (e.g. cell extension, branching pattern) until adulthood. The observed developmental changes of astroglial cells may contribute to the known delayed maturation of potassium regulation in rat hippocampus.

STRUCTURAL CHARACTERISATION AND BINDING PROPERTIES OF THE EXTRACELLULAR MATRIX PROTEIN RESTRICTIN

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Restrictin is a chick extracelluar matrix glycoprotein expressed by neurons and glial cells.
cDNA cloning revealed that restrictin shows substantial sequence similarity with tenascin. The predicted protein sequence of 1.353 amino acids reveals that restrictin is a large multidomain protein composed of four structural motifs. At the N-terminus restrictin contains a cysteine-rich segment of about 140 aa that might link restrictin monomers into oligomers. This region is followed by four and a half EGF-like repeats and then by nine consecutive motifs that are similar to fibronectin type III motifs. At the C-terminus restrictin is related to the β-and γ-chains of fibrinogen including similarity to a calcium-binding segment.
To identify binding proteins of restrictin and to localize binding activities within the restrictin molecule, we have produced a set of fusion proteins consisting of glutathione-S-transferase linked to one or more domains of fibronectin type III repeats. A cell attachment site of restrictin for an unknown cellular receptor was mapped to the C-terminal region of restrictin. Distinguishable of this cell binding site an interaction with the axonal surface glycoprotein F11 was localized within the FN type III repeats of restrictin.

SELECTIVE GLIOVASCULAR LESIONS PRODUCED BY *m*-DINITROBENZENE IN THE RAT BRAINSTEM.

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M-dinitrobenzene is one of a group of neurotoxic chemicals which damage astrocytes, oligodendrocytes and endothelial cells in the brain[1]. Following systemic administration of *m*-dinitrobenzene, lesions develop within a number of brainstem areas, notably the cerebellar roof and vestibular nuclei and the inferior colliculi. When examined at 12 to 24 hours these lesions are characterised by vacuolation of astrocytes and oligodendrocytes, withdrawal of astrocytic end feet, and by focal breakdown of the blood-brain barrier. Neuronal damage is not seen at this stage. At later times secondary neuronal death is seen in the more severe lesions, presumably due to the lack of glial support. The reasons for the selective vulnerability of glial cells to these toxic agents is not yet clear, but since lesion severity can be modulated by increasing or decreasing local functional activity within the brain[2], it is likely that an interference with glial metabolic capacity is involved.
We suggest that administration of dinitrobenzene provides a means of examining the consequences of loss of glial cell function in the intact brain, and also for looking at glial cell repair and proliferation.

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SOLUBLE GROWTH FACTORS STIMULATE THE SYNTHESIS OF SPECIFIC GLYCOSAMINOGLYCANS IN CULTURED RETINAL MÜLLER GLIAL CELLS.

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Retinal Müller glial (RMG) cells were isolated and cultured according to previously published procedures. Confluent 2nd passage cells were incubated in defined medium containing ^{3}H-glucosamine, with or without the addition of basic fibroblast or epidermal growth factor (bFGF or EGF respectively) for 48h. Samples were collected from both the soluble (medium) and insoluble (cellular) compartments, and glycosaminoglycans (GAG) were purified by enzymatic and alkaline digestion, the non-digested peptides being precipitated by sequential treatment with TCA and ethanol. The different GAG (Heparan Sulphate: HS; Chondroitin Sulphate:CS; Hyaluronic Acid: HA) were identified and quantified by specific enzyme digestion. In all cases, secreted GAG greatly outweighed the insoluble fraction, with 89% total GAG in this fraction. The distribution of GAG in the different cultures was: controls - 14% HS: 65% CS: 21% HA; bFGF - 11% HS: 56% CS: 33% HA; and EGF - 13% HS: 46% CS: 41% HA. On a unit protein basis, bFGF stimulated HS and CS levels 5 fold, and HA levels 10 fold; EGF stimulated HS levels 4 fold, CS 3 fold and HA 8 fold. These results indicate that i) the predominant GAG in cultured RMG is CS, which may be related to high CS levels in extracellular compartments within the retina, suggesting these cells as a possible source; ii) the relative increase in HA is probably related to extracellular matrix remodelling in the glial migration and proliferation induced by these two mitogenic growth factors. However, the presence of high levels of HA in retinal extracellular compartments and the existence of a HA receptor on apical regions of RMG cells in vivo suggests that HA may have a special role in these glial cells.

Expression of full length trkC during embryonic and postnatal development of the rat sciatic nerve

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Schwann cells are able to produce *in vitro* and *in vivo* in response to lesion and during development of the nerve a variety of neurotrophic factors. In order to investigate whether neurotrophic factors could possibly act in an autocrine fashion on Schwann cell proliferation and/or differentiation, we decided to analyze the expression of the high affinity neurotrophin receptors in the rat sciatic nerve. Neither trkA nor full length trkB could be detected at any time point. In contrast, truncated trkB mRNA and protein was found in the sciatic nerve through development up to adulthood. Using western blotting techniques we were able to show that full length trkC, the receptor for NT3 is expressed in the sciatic nerve of newborn rats as well as truncated trkC. Northern blots reveal that full length trkC is expressed as early as E16, but is downregulated in the adult animal. Interestingly, we could not detect reexpression of full lenght trkC after lesion of the adult sciatic nerve. RT-PCR combined with sequencing showed that both trkC and the nonfunctional trkC14 which contains an 14 amino acid insertion in the tyrosine kinase domain are expressed in the sciatic nerve from embryonic day 16 on and are still expressed by postnatal day 21. These findings indicate tat NT3 might play an important role in Schwann cell development.

TRANSPLANTATION STUDIES IN A MODEL OF SPINAL CORD TRAUMA IN THE RAT.

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Photoactivation of the intravenously administered dye, Rose Bengal, results in intravascular free radical production. This principle has been used to produce a model of spinal ischaemia in the rat in order to perform transplantation studies aimed at preservation and regeneration of damaged CNS tissue without causing paralysis.

The lesion has been studied using light and electron microscopy. Vascular thrombosis occurs at the centre of the lesion and is surrounded by an area of cellular death and axonal disruption. This extends over the subsequent seven days to occupy the dorsal funiculus and dorsal horns. The necrotic debris is cleared by macrophages over the next three weeks to leave a cyst lined by astrocytes and meningeal cells one month after lesion production.

Mixed glial cells prepared from neonatal rats have been transplanted into three day old lesions and consistently produce a dense astrocytic scar that is concentrated towards the poles of the lesion. This is in marked contrast to transplanted fetal cell suspensions that produce neurones within the lesion. These grow to such an extent that the surrounding normal spinal tissue is compressed.

GLIOSIS, DOES IT NATURALLY OCCUR IN THE OPTIC NERVE OF THE ADULT CAPE MOLE RAT (GEORYCHUS CAPENSIS)?

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The term "gliosis" is used to describe proliferation and hypertrophy of astrocytes in immediate areas of damaged central nervous tissue (CNS). The activated astrocytes then form a glial scar. There is conflicting evidence concerning the role of the scar regarding regeneration and failure of regeneration in the CNS, respectively. Some investigations show how reactive astrocytes form an obstacle to the growth of axons whereas other studies document reactive astrocytes as a permissive substrate that promote, rather than inhibit axonal growth. - Surprisingly the optic nerve of the naturally blind mole rat shows several features common to those observed in degeneration/regeneration of adult mammalian CNS : (i) glial cells appear in clusters composed of 5-7 cells; (ii) these cell clusters, as well as the axons, are separated from each other by larger and extended astrocytic cell processes, containing bundles of densely packed gliofilaments; (iii) at the level of the external glial limiting membrane a duplication of the basement membrane is present; (iv) a relatively high number of axons remains unmyelinated; these axons show a high variability in diameter. Investigations regarding the structural organisation of other parts of the visual system, as well as a developmental study of the mole rat, will provide new and relevant information on the reciprocal communication of neurons and glia. Supported by MRC and ERC grants.

PATCH-CLAMP STUDY OF A DELAYED RECTIFIER K^+ CHANNEL IN GUINEA PIG MÜLLER GLIAL CELLS

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Müller cells are the principal glial cells of the retina. By spanning the whole retinal thickness they contact all neuronal layers and appear to be involved in K^+-homeostasis. Although the Müller cell membrane is mainly permeable to K^+, its features are far from being well characterized. Because of the large membrane area, whole-cell experiments have several disadvantages. Therefore, we used the cell-attached configuration of the patch-clamp technique to record currents from patches of enzymatically isolated Müller cells. We used patch pipettes with resistances of more than 20 MΩ. Nevertheless, macroscopic outward currents were evoked by depolarizing steps. The characteristics of these currents resembled delayed rectifier currents. The K^+-concentration in the pipette solution as well as in the bath solution was 3 mM. Under these conditions 17.7% (n=62) and 39.5% (n=86) of the patches in the soma and endfoot region, respectively, contained delayed rectifier currents. Principally they were also found on the distal and proximal main process of the cell (n=3). The amplitude of the outward current differed from patch to patch and ranged from 5 to 65 pA (90 mV depolarization). These results point out that the delayed rectifier channels could be clustered together whereas the clusters seem to be nonuniformly distributed about the cell membrane. The delayed rectifier activated at membrane potentials more positive than -30 mV. Half-maximal activation was reached at about +15 mV. Steady state inactivation was still incomplete at +30 mV. However, the function of this channel remains unclear at present.

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IN THE CNS PMP 22 mRNA IS SELECTIVELY EXPRESSED IN MOTONEURONS.

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In the PNS, peripheral myelin protein PMP22 is expressed by Schwann cells and is a constituant of myelin. Point mutations in the mouse *PMP22* gene were shown to be responsible for the trembler and trembler-j phenotypes. In human, a duplication or deletion of one megabase region including the *PMP22* gene has been shown to be involved in Charcot-Marie-Tooth type Ia disease and in tomaculous neuropathy, respectively. In the rat, two populations of *PMP22* transcripts, SR13 and CD25, differing by their 5' non coding sequence have been described. PMP22 mRNA has been reported to be present in the CNS although at a much lower level than in the PNS.

The aim of this study was to localize the PMP22 expressing cells in the CNS. In a first series of experiments, we investigated by RT-PCR the presence, in different regions of the mouse brain, of PMP22 mRNA using a set of oligonucleotide primers specific for SR13 and CD25 transcripts, respectively. SR13 mRNA was detected in the cortex, diencephalon, brain stem and cerebellum. In contrast, both CD25 and SR13 were present in the spinal cord.

The cellular localization of PMP22 transcript in mouse CNS, was investigated by in situ hybridization with [^{35}S] radiolabelled cRNA probes. On 21 day-old animals, a PMP22 signal was selectively observed in the motor nuclei of the cranial nerves: trigeminal (V), fascial (VII), ambigus (IX), vagus (X) and hypoglossal (XII) nerves. The PMP22 signal was very weak in the occulomotor (III) and trochlear (IV), and absent from the abducens nucleus (VI). This pattern of localization, as well as the morphology of the $PMP22^+$ cells, strongly suggested that the corresponding transcript was expressed by neuronal cell bodies. In the spinal cord, only the motoneurons of the ventral horn were labelled with the PMP22 probe. The neuronal expression of PMP22 was confirmed by combined in situ hybridisation, using digoxigenin-labelled PMP22 cRNA probe, and immunohistochemistry, using cell-type-specific antibodies. The physiological implication of PMP 22 mRNA expression in a subset population of neurons involved in motor controls will be discussed.

Glycine and GABA induced currents in glial cells of the rat spinal cord slice

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In a recent study four types of glial cells could be distinguished in the rat spinal cord slice using electrophysiological and morphological methods. Astrocytes, oligodendrocytes and two types of precursor cells showed a distinct type of current pattern. In the present study, we demonstrate that these cells respond to the inhibitory neurotransmitters glycine and GABA, as revealed with the whole cell recording configuration of the patch clamp technique. All astrocytes and glial precursor cells and a subpopulation of oligodendrocytes responded to glycine. Similarly, large GABA currents could be elicited in astrocytes and precursor cells while oligodendrocytes showed only small responses. The GABA-activated current is due to the activation of $GABA_A$ receptors since the reversal potential was close to the Cl^- equilibrium potential. The response could be reversibly blocked by bicuculline and mimicked by muscimol. The involvement of glycine receptors is infered from the observation that the response was blocked by strychnine and that it reversed close to the Cl^- equilibrium potential. Besides of the activation of the Cl^- current, $GABA_A$ receptor activation also induced a block of the resting K^+ conductance, similar as observed in Bergmann glial cells. The presence of the glycine receptor seems to be unique for spinal cord glial cells since such responses have not yet been described in any other preparation. This further implies that such glial receptors may be involved in synaptic transmission since glycine is a dominant inhibitory neurotransmitter of the spinal cord.

GLUTAMATE PRODUCTION BY RAT AND MOUSE MICROGLIA: SPECIES DIFFERENCES AND EFFECT OF ENDOTOXIN, HIV COAT PROTEIN gp120 AND DEPOLARIZING AGENTS

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Rat and mouse microglia subcultures grown in a serum-free, glutamine-free defined medium, released only minimal amounts of glutamate over a 6 h incubation period. In the presence of a physiological concentration of glutamine (0.5 mM) glutamate release was substantially increased. Moreover, mouse microglia was found to contain and release more glutamate than rat microglia. Since glutamate reuptake was virtually absent in microglia, glutamate accumulation in the culture medium increased with time and with cell density. For example, when 10^6 mouse microglial cells were cultured in 0.4 ml of medium in 2 cm^2 wells for 6 h, the concentration of glutamate in the medium rose to about 60 mM in 6 h. A variety of microglia-activating or depolarizing agents, used to simulate pathophysiological conditions [endotoxin (10-100 ng/ml), HIV coat protein gp120 (0.1-10 nM), high K^+ (35 mM) or ATP (150mM)], did not alter rat or mouse microglial glutamate release when the cells were cultured in serum-free medium. In the presence of 1% fetal calf serum, however, 100 ng/ml of endotoxin caused a 2-3 fold increase in the accumulation of glutamate in the medium in a 6 h period. It is concluded that microglia-derived glutamate might contribute to microglial neurotoxicity in those pathological conditions in which intense local recruitment of activated microglial cells takes place.

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CULTURED AMOEBOID MICROGLIA RETAIN HIGH PROTEASE NEXIN-1 IMMUNOREACTIVITY

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A glia-derived neurite-promoting factor with serine protease inhibitory activity named protease nexin-1 (PN-1) was recently found to be expressed, in addition to glial cells, in distinct neuronal populations of the rodent brain. Further in vivo studies combining PN-1 immunocytochemistry and staining with microglial marker lectin revealed that the macrophage-like amoeboid microglial cells also contained PN-1 immunoreactivity. The amoeboid microglia are known to be localized in so called "hot spots" in the developing brain, e.g. the cingulum, external and internal capsule, and would play a major role during development of these neurite tracts. Primary cultures of microglial cells prepared from neonatal rat brain and identified by the specific marker isolectin B4 also showed PN-1 immunoreactivity on immunoblot and by immunocytochemistry. In the microglial cells, PN-1 immunoreactivity was found in large granules filling a substantial part of the cytoplasm. Surprisingly, Northern blot analysis of cultured microglia did not reveal the PN-1 mRNA in these cells. This observation would suggest that microglial cells are able to synthesize PN-1 while its mRNA level is present at very low level or unstable. Alternatively, microglia could internalize PN-1 protein present in their environment. The presence of PN-1 may help these cells to cope with serine proteases such as plasminogen activator(s) which were recently shown to be secreted by cultured microglia (Nakajima et al, Brain Res 577, 285, 1992).

GLUTAMATE UPTAKE BY ASTROCYTES: A MECHANISM LINKING FUNCTIONAL ACTIVITY TO ENERGY METABOLISM

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Astrocytes play a central role in brain energy metabolism. For example glucose, the exclusive brain energy substrate, is avidly taken up by astrocytes (PNAS 90:4042, 1993). Application of glutamate (Glu), the main excitatory neurotransmitter, stimulates in a concentration-dependent manner ^{3}H-2-deoxyglucose (2-DG) uptake by astrocytes in primary culture with an EC_{50} of ~ 100 µM. The effect is not receptor-mediated since it can not be reproduced by glutamatergic agonists (such as NMDA, kainate, quisqualate, t-ACPD) nor prevented by antagonists (APV, CNQX, AP3). Instead, it involves glutamate uptake since the effect is blocked by either DL-threo-β-hydroxyaspartate or L-CCG III, two potent inhibitors of the Glu transporter. Replacement of Na^+ in the medium by choline also prevents the effect, suggesting a Na^+ driven process. Finally ouabain, a selective inhibitor of the Na^+/K^+ ATPase, completely prevented the effect of Glu. These observations suggest that when glutamatergic synapses are active, glutamate, taken up by astrocytes, stimulates glucose uptake by astrocytes whose end-feet are known to surround capillaries, i.e. the source of glucose. They also provide a simple explanation for the mechanism linking functional activity to energy metabolism.

DEVELOPMENTAL DIFFERENCES OF GFAP-mRNA-EXPRESSION IN RAT HIPPOCAMPUS AFTER PRE- AND POSTNATAL LEAD EXPOSURE

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In this study we used in situ hybridisation to examine the effects of chronic low level lead toxicity during different periods of brain development. Low level lead is known to affect astroglia. GFAP (glial fibrillary acidic protein) was chosen as glialtypic marker for neurotoxicity. The effects of lead were investigated on male Wistar rats. Animals were divided into four groups: a control group, a permanent group exposed during and after brain development (E0-P100), a perinatal group exposed only during brain development (E0-P16) and a post-weaning group exposed after brain development (P16-P100). All experimental animals were fed a diet containing 750 ppm lead acetate. A radioactive mRNA in situ hybridisation was performed on frozen sections.
Significant differences to controls could be detected in the post-weaning group (exposure: P16-P100) where we observed a strong increase of GFAP-mRNA in the polymorphic zone of the dentate gyrus and in the region CA1 of the hippocampus. Permanent and perinatal group showed no overt changes compared to controls. Ultrastructural and immunocytological investigations with an GFAP-antibody confirmed these results. Our findings suggest that an irritation of the mature astrocyte results in a change from the quiescent to the reactive state. The majority of astrocytes that have been exposed during their development and differentiation fail to react even if the exposure is continued to adulthood. This indicates an irreversible insult to the immature astrocyte by low level lead exposure during this period of time.

VOLTAGE-DEPENDENT CA^{2+} FLUXES INTO LEECH RETZIUS NEURONES AND GLIAL CELLS

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Changes in the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) caused by the depolarization of leech Retzius neurones and neuropile glial cells were measured by using iontophoretically injected Fura-2. In physiological Ringer solution the $[Ca^{2+}]_i$ of Retzius neurones was 78 nM $\pm$ 34 nM and that of glial cells 92 nM $\pm$ 58 nM).

In both Retzius neurones and glial cells[1] a rise in the extracellular K^+ concentration ($[K^+]_o$) evoked an increase in $[Ca^{2+}]_i$ by up to 2 µM ($[K^+]_o$ = 89 mM, extracellular Na^+ completely replaced by K^+). This increase is due to the activation of voltage-dependent Ca^{2+} channels, because raising $[K]_o$ in the absence of extracellular Ca^{2+} had no effect on $[Ca^{2+}]_i$. Furthermore, the K^+-induced $[Ca^{2+}]_i$ increase was reversibly blocked by mM amounts of Ni^{2+} or Co^{2+}, as well as by menthol, which has been shown to inhibit voltage-dependent Ca^{2+} currents in cultured dorsal root ganglion cells[2]. At elevated $[K^+]_o$ levels $[Ca^{2+}]_i$ was steadily increased which suggests that the voltage-dependent Ca^{2+} channels do not inactivate.

In both cell types $[Ca^{2+}]_i$ increased only when $[K^+]_o$ was raised to more than 10 mM ($[K^+]_o$ = 4 mM in Ringer solution). The plot of the $[Ca^{2+}]_i$ increase versus the membrane potential recorded at the different $[K^+]_o$ levels showed that in Retzius neurones a $[Ca^{2+}]_i$ increase occurred when the membrane potential was more positive than -30 mV, which closely corresponds to the threshold of the voltage-dependent Ca^{2+} current determined in voltage-clamp experiments[3,4]. In glial cells a depolarization to -50 mV was sufficient to elicit a detectable $[Ca^{2+}]_i$ increase.

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HUMAN MEDULLOBLASTOMA CELL LINES AS A MODEL FOR NEURAL PROGENITOR CELLS

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Medulloblastoma (MB) represents the most frequent malignant brain tumor of childhood, but only few cell lines and animal models of this primitive neuroectodermal tumor (PNET) have so far been established. Using specific cell culture conditions, we were able to derive four human MB cell lines (MHH-MED-1 to -4). The four MB cell lines grew in suspension as floating cell aggregates or as slightly adherent cells. They consisted of undifferentiated cells that did not express markers of late neuronal or glial lineages such as neurofilaments or GFAP. They also lacked expression of MHC class I or II antigens on the cell surface. All four MB lines were positive for vimentin and neuron-specific enolase, whereas synaptophysin, N-CAM, GAL-C, GD2, GD3, and the A2B5-antigen were expressed inconsistently. By cytogenetic analysis, the lines were near diploid with clonal aberrations. The MB lines showed no losses of chromosome arm 17p by either cytogenetic or microsatellite analyses. The cell line MHH-MED-2 exhibited double minute chromosomes, amplification of the c-*myc* gene, and overexpression of c-*myc* mRNA and protein. N-*myc*, *mdm2*, p53 and Rb1 protein expression were unaltered. All four continuously passaged MB cell lines were subcutaneously xenotransplanted into athymic mice and three of the MB lines gave rise to tumors. These cell lines will be useful tools for biological studies on PNETs and a model for human neural progenitor cells.

ASTROCYTES *IN SITU* RESPOND TO NEUROLIGANDS AND DEPOLARIZATION

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It is becoming increasingly clear that astrocytes play very dynamic and interactive roles which are important for the normal functioning of the central nervous system. In culture, astrocytes express many of the same receptors as neurons. *In vivo,* it is likely that these receptors are important for the modulation of astrocytic functions such as the uptake of neurotransmitters and ions. Currently, however, very little is known about the expression or stimulation of astrocytic receptors *in vivo*. Using confocal microscopy and calcium sensitive fluorescent dyes we obtained data indicating that hippocampal astrocytes *in situ* (identified by glial fibrillary acidic protein immunostaining) respond to glutamate, 1-aminocyclopentane-trans-1,3-dicarboxylic acid (t-ACPD), N-methyl-D-aspartate (NMDA), ATP, and high K+ with increases in intracellular calcium ([Ca2+]i). The responses exhibited various temporal patterns which included both sustained plateaus and oscillations. Increases in [Ca2+]i occurred both in the cell bodies and in the processes and were unaffected by 1 uM tetrodotoxin. Often a single astrocyte responded to more than one neuroligand and to depolarization. These findings suggest that astrocytes *in vivo* contain receptors coupled to increases in [Ca2+]i and are able to respond to neuronally released neurotransmitters. The presence of multiple pathways for increasing [Ca2+]i within a single astrocyte suggests the possibility of integration of multiple stimuli.

PROTEIN KINASE C-ACTIVATING TUMOR PROMOTERS INDUCE A MITOGENIC RESPONSE IN DIFFERENTIATED OLIGODENDROCYTES

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Aggregating brain cell cultures prepared from 16-day rat embryos attain a high degree of cellular organization and differentiation. Within about 4 weeks *in vitro*, the two oligodendroglial paramaters, 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) activity and myelin basic protein (MBP) content attain maximal values, in good agreement with morphological findings indicating the presence of myelinated axons. After a single treatment of these cultures at day 26 with a specific protein kinase C-activating tumor promoter (either 50 nM of phorbol 12-myristate 13-acetate or 50 nM of mezerein), thymidine incorporation was significantly increased at day 30, and accompanied by a drastic decrease in total MBP content and CNP activity. At day 46, both the mitotic activity and the two oligodendroglial differentiation markers were reversed to (or beyond) the levels of control cultures. In the presence of basic fibroblast growth factor (5 ng/ml), the mitogenic response as well as the decrease and subsequent recovery of MBP content and CNP activity were significantly enhanced. These results suggest that the stimulation of protein kinase C induced myelinating oligodendrocytes to dedifferentiate and to proliferate, and that the responding (and the newly generated) oligodendrocytes were able to attain again a high level of differentiation. Furthermore, basic fibroblast growth factor appears to potentiate the response of mature oligodendrocytes to protein kinase C-activating tumor promoters.

bFGF AND ITS RECEPTORS IN HUMAN FETAL MICROGLIA

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We studied the expression of bFGF and its receptors in several lines of human fetal microglia. Expression and production of bFGF were assayed through Northern blot analysis, immunocytochemistry, biological assay and Western blot analysis. Results showed that all cell lines tested produced functionally active bFGF. The factor was retained inside the cell, as already shown in several cell types, including human neurons. The presence of different types of bFGF receptors was also assayed with ^{125}I-bFGF binding, Northern blot analysis and immunocytochemistry. Scatchard plot analysis of binding data demonstrated the presence of 10,300 ± 2,500 high affinity sites per cell (Kd = 30 ± 9 pM). Low affinity binding sites were approximately 380,000 ± 60,000/cell (Kd = 730 ± 200 nM). Northern blot analysis confirmed the expression of FGF receptor-1/flg. Colocalisation of FGF and FGF receptor performed by immunocytochemistry showed positive staining in some, not all round-shaped cells, presumably ameboid microglia. The physiological significance of bFGF on ameboid and ramified microglia is also investigated.

NEUROPEPTIDES AND ATP INDUCE IMMEDIATE EARLY GENE TRANSCRIPTION IN ASTROCYTES AND MICROGLIA

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Neuronal injury leads to characteristic changes in surrounding microglia and astrocytes but the signals underlying the glial responsiveness are poorly understood. Possible mediators between damaged neurons and glia are the neuropeptides CGRP and galanin, both of which are upregulated in motoneurons after axotomy. A further candidate is ATP which is released from injured or dying cells and has been shown to activate ion fluxes in cultured microglia. To elucidate further the role of CGRP and galanin as well as ATP as signalling molecules, we investigated their effects on immediate early gene (IEG) expression in cultures of astrocytes and microglia. CGRP led to a rapid and transient induction of c-fos and junB mRNA in cultured astrocytes, whereas c-jun and TIS 11 mRNA levels were unaltered. Galanin induced c-fos, junB and TIS 11 mRNA with a time course differing from that of CGRP. Therefore, astrocytes possess specific receptors for both neuropeptides and the differential induction of IEG mRNAs points to differences in intracellular signalling events. Cultured microglia also responded to CGRP with an upregulation of c-fos and junB mRNA. No changes in c-jun or TIS 11 mRNA levels were observed. In contrast to astrocytes, galanin had no effect on c-fos expression in microglia. While ATP and ADP were almost equipotent at increasing c-fos mRNA in cultured microglia, AMP and adenosine showed only weak effects. No induction of c-fos mRNA was observed with the P_{2X}-agonist β,γ-methylene-ATP, but the P_{2Y}-agonist ADPβS led to a time-dependent increase in c-fos, junB, c-jun and Tis 11 mRNA. Therefore the effects of ATP on early response gene expression in microglia are presumably mediated via a P_{2Y}-receptor. In conclusion, CGRP, galanin and ATP differentially induce a number of immediate early genes in cultured glial cells. The data suggest a role for neuropeptides and ATP in the transcriptional activation process of both astrocytes and microglia.

AXONAL GROWTH ON MYELIN AND MEMBRANES FROM OPTIC AND SCIATIC NERVES

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Glial cells can exhibit either growth-promoting or inhibitory influences on growing axons. The ability of peripheral axons of mammals to regrow after lesions has been explained by the supportive role of Schwann cells for neuronal survival and axon growth. In contrast, in the central nervous system (CNS) axons show only abortive sprouting and do not grow over longer distances. This observation has been explained by inhibitory aspects of mature oligodendrocytes and CNS myelin which is produced by these cells. PNS myelin, synthesized by Schwann cells, has been regarded as a permissive substrate for regenerating axons. In the present study we examined the role of CNS and PNS myelin and myelin free membranes on axon growth. Myelin and myelin-free membranes were prepared from adult rat optic or sciatic nerves. Membrane fractions were separated on a sucrose density gradient and after purification the protein pattern was documented on Western blots. Purified membranes from optic and sciatic nerves did not contain visible amounts of myelin. Both myelin and membranes from either optic or sciatic nerves were used as substrates for retinal ganglion cell (RGC) axons from embryonic chick and rat. Our results show that CNS and PNS myelin fractions alone are not permissive for the growth of RGC axons. On purified myelin free membranes from normal and axotomized optic nerves consistent outgrowth could be obtained. Precoating with myelin allowed sparse RGC axon growth. This suggests that inhibitory aspects of myelin might not only be found in the CNS but also in the PNS. The ability of individual axon populations to regrow in situ might therefore be determined by upregulation of growth promoting molecules.

CELLULAR IMMUNE RESPONSES FOLLOWING UNILATERAL AND BILA - TERAL GRAFTS OF ALLO - AND XENOGENEIC ORIGIN IN THE 6-OHDA MODEL OF THE RAT

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We investigated the influence of immunogeneic xenografts on function and survival of cografted non - immunogeneic allotransplants in unilateral, 6 - hydroxydopamine (6-OHDA) lesioned rats. Additionally we studied the effect of Cyclosporine A (Cy A) on the immune response. 72 lesioned rats were transplanted with cell suspensions of foetal ventral mesencephalon (VM) and fetal striatum (STR) according to the following design: *group 1*: lesioned striatum mixed VM and STR (rat, allogeneic tissue), no CyA; *group 2*: lesioned striatum VM (rat); non-lesioned striatum STR (rat); no Cy A; *group 3*: lesioned striatum mixed VM (rat) and STR (mouse, xenogeneic tissue), no Cy A; *group 4:* lesioned striatum mixed VM (rat) and STR (mouse, xenogeneic tissue), Cy A treatment for 42 d; *group 5*: lesioned striatum VM (rat); non-lesioned striatum STR (mouse), no Cy A; *group 6*: lesioned striatum VM (rat); non-lesioned striatum STR (mouse), Cy A treatment for 42 d; *group 7*: lesioned striatum sham transplantation (saline), no Cy A; *group 8*: lesioned striatum sham transplantation (saline), Cy A treatment for 42 d. The function of the graft was examined in-vivo with D - amphetamine - induced rotation testing. Rotation data 42 d post grafting revealed significant decreases in ipsilateral circling as compared to pregraft levels in group 1, 2, 3, 4, 5 and 6 (paired Student´s t-test, $p<0.05$) but not in the sham-groups 7 and 8. Six weeks post grafting the animals were perfusion fixed, and the brains were processed with immunocytochemistry for tyrosinehydroxylase immunoreactive (TH - IR) grafted cells, mouse tissue, microglia, astrocytes, T - lymphocytes and macrophages. The number of intrastriatal TH - IR cells in group 3 and 5 without Cy A treatment was lower, but not significantly reduced compared to group 1, 2, 4, and 6 (ANOVA, Scheffé Test n.s.). Surviving mouse tissue was found in animals with immunosuppression only (group 4 and 6). The number of microglial cells, astrocytes and macrophages was higher in animals with a xenograft component and independent from immunosuppressiv treatment. The number of T-lymophcytes was reduced within the allograft and under Cy A treatment. These results suggest that in the CNS continued immunosuppression is necessary for function and survival of mixed neuronal cografts combining allo - and xenogeneic tissue. The rejection of a xenogeneic graft component may compromise survival and function of the cografted allogeneic tissue, when grafted in a mixed cellsuspension. Supported by the German Federal Ministry of Research and Technology.

USE OF ENDOTHELIAL CELLS AS GENE CARRIERS IN BRAIN REPAIR ATTEMPTS

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The introduction in a deficient brain of foreign cells genetically manipulated to express a transgene may be useful to restore a proper functioning of the grafted brain. Fibroblasts have been used for that purpose by several groups. However, utilization of cells which are normally present in brain parenchyma would probably improve the efficiency of this therapy by reducing undesirable side-effects due to the presence, in the grafted brain, of foreign implanted cells. Immortalized non-tumorigenic endothelial cells (RBE4) from rat brain microvessels have been manipulated to express the lacZ gene in a preliminary approach to gene therapy. These cells have been found to survive and express the transgene for several weeks after implantation in adult rat brains. They tended to associate with host endothelial cells with which they made specialized connections such as desmosomes and tight junctions. Preliminary experiments where the gene encoding for the NGF was introduced in the RBE4 cells showed immunohistochemically detectable NGF expression at the site of implantation and at a distance where implanted cells had migrated.

THE L-GLUTAMATE TRANSPORTER OF RETINAL GLIAL CELLS IS NOT IMMUNOLOGICALLY RELATED TO THE L-GLUTAMATE TRANSPORTER OF BRAIN GLIAL CELLS

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The major part of the excitatory neurotransmission in the mammalian central nervous system (CNS) is mediated by L-glutamate. Termination of such neuronal activity is achieved by two sodium-dependent high affinity uptake systems, one being located in astroglial cell processes, the other one in presynaptic nerve endings. Both transport systems appear to be of clinical relevance since they represent mechanisms by which synaptically released glutamate is inactivated and kept below toxic levels.

The retina provides an excellent model tissue for investigating the pharmacological and molecular properties of both transporter systems since glutamate is the excitatory transmitter used by photoreceptors, bipolar cells and ganglion cells. Conventional astroglia and microglia are present in the retina, but by far the most predominant cell type are the large and radially orientated Müller glia cells. Autoradiographic studies in the retina show that glial uptake of L-glutamate is much stronger than neuronal uptake, similar to the situation observed in the cortex. Comparative immunoblot analysis using polyclonal antibodies against the purified glial L-glutamate transporter of rat brain exhibits a strong band at about 72 kDa for probes from cortical brain tissue derived from different species. However, the same antiserum labels only weakly a protein band of similar molecular weight in retinal probes of the same species, in spite of the massive presence of Müller glia cells in the retina. Immunohistochemical labeling of retinal cryostat sections with the above mentioned antibodies specifically labels only a subtype of photoreceptor and bipolar cells whereas no labeling of either Müller cells nor astroglial cells is observed. These results indicate that the glutamate transporter proteins of cerebral glial cells and retinal glial cells are not identical proteins. Further pharmacological and kinetic studies will be performed to look for differences between glial L-glutamate transporter in cortex and retina.

DIFFERENTIAL EXPRESSION OF TISSUE PLASMINOGEN ACTIVATOR AND PLASMINOGEN ACTIVATOR INHIBITOR-1 IN NEURONS AND GLIA FOLLOWING MOTONEURON INJURY

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Plasminogen activators are proteases that have been implicated in tissue repair and remodelling processes. However, very little is known about the reaction of the plasminogen activator system following injury of the nervous system. Here we report studies on the reaction of tissue plasminogen activator (tPA) and the plasminogen activator inhibitor-1 (PAI-1) in the rat facial nucleus to axotomy of the facial nerve. An increase in motoneuronal tissue plasminogen activator (tPA) immunoreactivity and tPA-mRNA occured after axotomy, reaching a peak after 3-5 days, then declining to control levels by 14 days. From 14 days to 8 weeks after axotomy, tPA immunoreactivity was also observed in astrocytes surrounding injured motoneurons. An increase in PAI-1 immunoreactivity was also found to occur 3 days after axotomy in perineuronal astrocytes. This increase was sustained at all times studied (up to 8 weeks). The results suggest a role for the plasminogen activator system in the cellular events accompanying transection and subsequent regeneration of motoneurons. Moreover, the fact that changes occur in tPA and PAI-1 in both the damaged neurons themselves and in astrocytes suggests an interplay between these cell types in mediating the cellular reorganisation of the facial nucleus following axotomy.

BRADYKININ-INDUCED $[Ca^{2+}]_i$ OSCILLATIONS IN RAT GLIOMA CELLS REQUIRE TRANSIENT HYPERPOLARIZATIONS OF THE PLASMA MEMBRANE.

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Oscillations of $[Ca^{2+}]_i$ which are associated with Ca^{2+} influx were investigated in rat glioma cells (C6-4-2). The $[Ca^{2+}]_i$ oscillations reversibly disappear at temperatures below 30°C, and the amplitude of the oscillations increases in the absence of extracellular Na^+. The oscillatory rise in $[Ca^{2+}]_i$ is synchronous with the hyperpolarizing phase of the peptide-induced membrane potential oscillation. Blocking the bradykinin-induced hyperpolarization by Ba^{2+} or by charybdotoxin abolished the $[Ca^{2+}]_i$ oscillation. Depolarization reduced, whereas hyperpolarization increased the amplitude of the $[Ca^{2+}]_i$ oscillations, indicating that the Ca^{2+} influx which occurs during the $[Ca^{2+}]_i$ oscillation is dependent on membrane potential. Thus, depolarizing stimuli possibly protect cells from a Ca^{2+} overload during oscillations.

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NA^+ CHANNELS ARE EXPRESSED BY MÜLLER (GLIAL) CELLS FROM VARIOUS MAMMALIAN SPECIES INCLUDING MAN

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Within the last few years, the expression of voltage-dependent, TTX-sensitive Na^+ channels has been demonstrated in several types of neuroglial cells such as astrocytes, Schwann cells, and enteric gliocytes. Here we describe the properties of Na^+ currents in Müller cells isolated from retinae of several mammalian species (4 dogs; 7 cats; 3 pigs; 8 mice; 2 zebras). Additionally, each one eyecup of two human postmortem donors were obtained after the corneae had been excised for transplantation. The Na^+ currents were eliminated by TTX (1 μM), and by exposure to sodium-free extracellular solution; their sign reversed close to the calculated E_{Na} of +65 mV. Voltage-dependent activation and inactivation characteristics and time constants of the Na^+ currents were similar to those of currents carried by neuronal Na^+ channels. The estimated number of sodium channels per cell was low (about 1 500 channels per 7 500 μm^2), and the K^+ conductance exceeded the peak Na^+ conductance by an average factor of 5. Modelling estimations show that triggering of glial Na^+ currents under physiological conditions, if any, can at best occur by ephaptic transmission at perinodal sites of optic axons. It is speculated that glial Na^+ channels might be involved in neuro-glial signalling events, as e.g. by activation of G-proteins. We are now studying the effects of elimination of specific neuronal cell populations (e.g., retinal ganglion cell loss after optic nerve section) onto expression of Na^+ channels by Müller cells.

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LIPOSOMAL-MEDIATED GENE TRANSFER TO HUMAN- AND RAT GLIOBLASTOMA CELLS

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mes or immunoGlioblastoma, the most common malignant brain tumor, is considered to be incurable. An innovative treatment strategy for tumor destruction is gene therapy. Successful gene transfer is a combination of delivery and expression of the foreign gene in the target cell. In our study we examine the value of liposomes and immunoliposomes as target specific delivery vehicles for marker or cytokine gene transfer in comparison to the retroviral vector system.

We used the reporter gene ß-galactosidase[LacZ] (p BAG) or retroviral vectors (pM3) (pWG29) carrying the hTNF cDNA for encapsulation in different types of liposomes and immunoliposomes (with anti glioma associated antigen; CLNIgG). The TNF expression after transduction of the vector pWG29 was inducible by dexamethasone (1 µM). These liposomes were tested on rat (F98)- and different human glioblastoma cells. The transfer efficiency in comparison to conventional methods with the retroviral vector was determined.

We can show that the liposomal transfer rate of marker-gene LacZ in various glioblastoma cells is comparable to conventional methods (0.5-5%) depending on the liposome type. Lipofectin, DC-chol- and immunoliposomes were selected for TNF gene transfer.

The evaluation of the gene transfer efficiency in vitro was the first step to develop an in vivo gene transfer method on the basis of liposomes or immunoliposomes for treatment of glioblastomas.

PRODUCTION OF RECOMBINANT FRAGMENTS OF NEUROCAN IN 293 CELLS AND NEUROCAN EXPRESSION PATTERNS IN HIPPOCAMPAL TISSUE SLICES.

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Neurocan is a chondroitin sulfate proteoglycan, which could be isolated from rat brain with the monoclonal antibody 1D1 (J. Biol. Chem. 266: 14785, 1991). It is a member of the aggrecan/ versican family of proteoglycans, and so far its message has only been detected in the central nervous system (J. Biol. Chem. 267: 19536, 1992). In functional assays with neuronal cell adhesion molecules it exhibited repulsive properties in aggregation and gravity adhesion assays and adhesive properties in centrifugation adhesion assays (J. Cell Biol. 120: 815, 1993).

To analyse in more detail, which structures are responsible for these effects and might be involved in the function of this proteoglycan, we have expressed C-terminal fragments of this proteoglycan, linked to the signal peptide of the basement membrane protein BM40, in the human embryonic kidney cell line 293, which is capable of expressing the appropriate neurocan fragments as chondroitin sulfate proteoglycans. Immunostainings of fresh hippocampal tissue slices show a colocalisation of the 1D1-epitope and GFAP, but the tissue slices display a complete change in the distribution of the 1D1-epitope after 4 days of cultivation.

EXPRESSION OF MYELIN PROTEINS IN CULTURED RAT BRAIN OLIGODENDROCYTES: INFLUENCE OF ORGANOTINS

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The growth and differentiation of oligodendrocytes and in particular the expression of myelin specific proteins, i.e. myelin basic protein (MBP), Wolfgram protein (WP), and proteolipid protein (PLP) was investigated in primary cultures of glia cells. Cultures were derived from the brains of newborn rats, incubated in normal growth medium and in the presence of trialkyltin derivatives, to assess the cellular and molecular events underlying organotin neurotoxicity.

The trialkyltin derivatives trimethyltin chloride, **TMT** and triethyltin chloride, **TET**, are highly neurotoxic. While TET has direct and specific effects on myelin formation in the brain. TMT causes localized neuronal damage.

Half maximal cytotoxicity after 24 h treatment was observed for TET at 0.7 μM and for TMT at 3.0 μM, as investigated by vital dye uptake, using neutral red. Indirect immunofluorescence studies showed that oligodendrocytes were particularly sensitive to TET treatment. The cell bodies were swelling and the cellular processes formed small membraneous droplets, before the cells were completely destroyed.

Western blot analysis indicated that in the presence of low concentrations of TET (0.1-0.3 μM) the amount of MBP was increased, while PLP and WP remained at the same level. At higher concentrations of TET (> 0.4 μM) and also of TMT (> 1.5 μM) a general decrease in myelin specific proteins was observed.

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REACTIVE GLIOSIS IN VITRO FOLLOWING CHEMICAL INJURY OF RAT BRAIN GLIA CULTURES

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Neuroglia respond to chemical or mechanical injury of the brain by a chain of events, including the activation of microglia, astrocytic hypertrophy and accumulation of glial fibrillary acidic protein (GFAP). The neurotoxic agent trimethyltin-chloride (TMT) in vivo causes neuronal damage, accompanied by an influx of microglia, induction of astrocytic proliferation and gliosis (Boyer (1989) Toxicology, 55, 253-298).

We have investigated the effects of TMT on rat brain glia cells in cultures derived from the cerebral hemispheres of newborn rats. Three weeks old cultures were subjected to TMT for 24 h at concentrations of 1.5-2.5 μM. In TMT treated cultures astrocytes altered their morphology and large holes appeared in the flattened cells. Indirect immunofluorescence staining revealed an accumulation and induced bundling of GFAP-containing intermediate filaments at lower concentrations (around 1-1.5 μM). At higher concentrations (2.5μM) the orderly array of intermediate filaments was disturbed and a diffuse but heavy staining with anti-GFAP antiserum was seen. The cellular holes were invaded by small cells of round appearance. These cells did not express GFAP, but contained tubulin, actin, S-100 protein and were labeled by the monoclonal antibody OX-42, showed phagocytotic activity and thus represented microglia cells. Quantitative evaluation indicated that TMT (2.0μM) caused a threefold increase in the number of microglia as compared to untreated control cultures. Thus, TMT directly affects glia cells in vitro even in the absence of neurons.

CHARACTERIZATION OF A NOVEL IMMUNOCYTOCHEMICAL MARKER OF FIBROUS AND REACTIVE ASTROCYTES

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During past years, efforts have been made to characterize molecules produced by reactive astrocytes that may be involved in the inhibition of axonal regeneration in the adult CNS. In this study, we have used a monoclonal antibody "6.17" that has been raised against embryonic rat muscle homogenate. The 6.17 immunostaining pattern was examined throughout control and lesioned brains of adult rats. Surgical lesions consisted in (i) stereotaxical lowering a metallic rectangular knife to the ventral surface of the diencephalon, (ii) complete section of the thoracic spinal cord. Double immunofluorescent labellings were performed on vibratome sections (40 µm thickness), using primary antibodies against 6.17, GFAP, vimentin and S100 protein, and secondary antibodies conjugated to fluorescein and rhodamine. In control animals, 6.17 immunostaining was observed in astrocyte-like structures located in the white matter, including optic tract, corpus callosum, fornix, and in the fasciculi of the spinal cord. Some discrete immunoreactive (IR) profiles were also detected in the peri-ependymal regions of both diencephalon and spinal cord, and in the spinal dorsal horn. All 6.17-IR profiles were S100 protein-, GFAP and vimentin-double labelled. In lesioned brains, a marked increased 6.17 immunostaining occurred in all regions surrounding lesion sites. In the diencephalon, 6.17 staining was associated with both GFAP- and vimentin-positive astrocytes closely surrounding the lesion. In the spinal cord, in close vicinity of the lesion site, 6.17 and vimentin double labelled profiles were detected in the white matter and throughout the entire spinal grey matter, including dorsal and ventral horns. This study indicates that the 6.17 antigen can be considered as a specific marker of fibrous and reactive astrocytes.

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SGP-2, A POTENTIAL MEDIATOR OF THE NEUROTROPHIC EFEFCTS OF MESENCEPHALIC GLIA ON DOPMAMINERGIC NEURONS

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Sulphated glycoprotein-2 (SGP-2) is a glucocorticoid-regulated 40 kD and 35 kD heterodimeric protein originally isolated from Sertoli cells of the testis. In the brain, SPG-2 is predominantly expressed by glial cell after injury or under certain pathophysiological conditions e.g. Alzheimer's disease. The function of SGP-2 in these degenerative processes is presently unknown. Our previous studies have demonstrated that a glucocorticoid-regulated protein with similar molecular weight may be involved in the survival-promoting effects of conditioned medium (CM) derived from both the mesencephalic glial cell line, Mes42, and from primary mesencephalic glia on cultured dopaminergic neurons. To test whether SGP-2 is secreted by Mes42 cells, Western blots of Mes42-CM were stained with SGP-2 antiserum*. Immunoreactivity was restricted to protein bands of the processed 35 kD and 40 kD SGP-2 subunits as well as to their 67 kD precursor protein. Moreover, inclusion of SGP-2 antiserum to serum-free cultures of the E15 mesencephalon specifically abolished the survival-promoting effects of Mes42-CM on dopaminergic neurons. In contrast to these results, partially purified SGP-2 did not support dopaminergic cell survival.

Together these findings suggest that SGP-2 may play a crucial role in mediating the survival-promoting effects of glial-derived growth factors on dopaminergic neurons and potentially acts as a carrier protein for these factors.

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QUANTITATIVE CONTRIBUTION OF RESIDENT MICROGLIA VS HEMATOGENOUS MACROPHAGES TO THE REMOVAL OF MYELIN DEBRIS IN ADOPTIVELY TRANSFERRED EAE

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The role of microglia in inflammatory brain lesions *in vivo* is as yet ill-defined. To investigate effector cell functions of resident microglia we performed quantitative light- and ultrastructural immunocytochemistry in (Lewis x BN) F1->BN rat radiation bone marrow chimeras. EAE was induced by i.v. injection of 1 x 10^7 MBP-reactive T blasts. Double labeling with I-1-69 (a mAB against Lewis MHC class II antigen) and ED1 (a mAB against lysosomal antigen) separated unequivocally bone marrow-derived cells (I-1-69 positive) from activated resident microglia (ED1 positive only).
Activated hematogenous macrophages (I-1-69pos/ED1pos) outnumbered activated resident microglia (I-1-69neg /ED1pos) 3 to 6-fold at both light- and ultrastructural level. Surprisingly, despite their smaller number, resident microglia contributed equally to the removal of myelin debris (50 % of all cells containing myelin degradation products).
Our results confirm that in acute brain inflammation the majority of brain macrophages are bone marrow-derived and indicate that the resident microglial cell population is extensively involved in phagocytosis of debris.

THE INITIAL SPIKE IN GLUTAMATE-INDUCED CALCIUM RESPONSES IN ASTROCYTES IS A RAPID INTERCELLULAR WAVE

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The mechanism of glutamate-evoked intracellular calcium responses in astrocytes is not elucidated yet. Intracellular calcium ($[Ca^{++}]_i$) was imaged in astrocyte cultures with Fluo-3 and a confocal microscope. Low magnification imaging of glutamate evoked responses over a field of 1.2mmx1.6mm showed that the initial spike of the response is not a spatially uniform $[Ca^{++}]_i$ transient but rather a change in $[Ca^{++}]_i$ propagating at an average speed of 390µm/s from cell to cell. The concentration gradient at the wave front was about 2000µm and the maximal $[Ca^{++}]_i$ at a particular location was reached within 3.6-5.4s. Based on its fast spatial component this initial $[Ca^{++}]_i$ transient was termed 'spatial spike'. In one experiment two spatial spikes propagated almost frontally toward each other and eventually collided. Their propagation velocity upon entrance in the field of view were lower (120µm/s and 85µm/s) than the velocity of spatial spikes observed otherwise and decreased within 14.4s to 20µm/s before they collided, suggesting that the spikes interacted. The rise time of the $[Ca^{++}]_i$ was reached for both spatial spikes within 3.6-5.4s but the measured length of the concentration gradient decreased as the propagation slowed down. During collision, the $[Ca^{++}]_i$ did not exceed the $[Ca^{++}]_i$ level of the individual spatial spikes, indicating that the mechanism of the spatial spike involves a reaction limiting step. The spatial spikes annihilated during collision without interfering with the immediately following $[Ca^{++}]_i$ oscillation or plateau. This suggests a refractory period for the spatial spike but not for the subsequent Ca^{++} response. Glutamate and t-ACPD, an agonist of the metabotropic glutamate receptor subtype, but not kainate evoked a spatial spike, indicating that the Ca^{++} raise during spatial spikes is linked to inositol-triphosphate (IP_3) production. Ca^{++}-free or Na^+-free superfusion solution and Benzamil, an inhibitor of the Na^+/Ca^{++}-exchanger, had only minor effects on spatial spikes. However, these treatments strongly affected the subsequent Ca^{++} responses, indicating that the spatial spike is generated by a different mechanism than its following $[Ca^{++}]_i$ oscillations and plateau. The initial spike of the glutamate-induced $[Ca^{++}]_i$ response in hippocampal astrocytes is a rapidly propagating intercellular wave probably mediated by IP_3-induced Ca^{++} release and thus based on a separate mechanism than its immediately following Ca^{++} responses including intracellular Ca^{++} waves. 705A-41-41983, 1-PO1-NS30619.

ASTROCYTES IN NEUROTRANSMISSION

Does an impairment of the astroglial regulation of extracellular glutamate and cell volume lead to the asteno-emotional syndrome?

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Astrocytes maintain ionic, amino acid and water homeostasis in brain extracellular space. The anatomy of the cells with their formation of network(s) together with their capacity to react upon and produce humoral and long-distance, slow speed transfer of information within the syncytium makes them appear as a class of cells with the capacity to produce integrated responses to multiple stimuli. Impairment of astroglial control of the extracellular milieu, *e.g.* glutamate (Glu) concentration, might lead to disturbances in neuronal excitability. This is the situation in several traumatic and toxic states. We propose an hypothesis that the asteno-emotional syndrome, often seen after slight brain trauma, infections or low-dose toxic exposure and characterized by fatigue, decreased simultaneous capacity and problems with concentration and memory, might be due to a glial impairment of the regulation of extracellular Glu. As a basis for our hypothesis we summarize late findings from our laboratory of receptor mediated signal transduction on the regulation of Glu carriers, different ion-channels and astroglial cell volume.

ASTROSTATINE, A PROTEIN SECRETED BY NEURONS, INHIBITS TYPE 1 ASTROCYTES PROLIFERATION.

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Our current understanding of gliogenesis has dramatically evolved with the findings of Raff and co–workers of several interacting glial populations (Science, 1989, 243, 1450). The type 1 astrocyte plays a key role in this scheme of gliogenesis. However, neurons, presents earlier in the development, don't seem to interact with this process and little is known about the regulation of type 1 astrocytes proliferation itself. We have reported that hippocampal or cerebellar granule neurons in culture release in their conditioned medium a protein which we have called Astrostatine and which reversibly inhibits type 1 astrocytes proliferation (J.Neurosc.Res., 1990, 25, 58). This inhibition is very selective since the proliferation of type 2 astrocytes, of microglia, of fibroblasts and of transformed astroglial cells is not inhibited by Astrostatine. Furthermore, this protein is only present in media conditioned by primary of transformed neuronal cells, not in media conditioned by normal or transformed astrocytes or fibroblasts. By gel filtration, we have estimated the molecular weight of this protein to be about 17 kDa. Astrostatine is different from several already known growth modulators such as TGF–ß, IFN gamma or TNF–α or –ß. We designed a purification scheme wich is based on the low solubility of Astrostatine in water and results in a preparation of Astrostatine showing as a single sharp band on SDS–PAGE. It starts with 3 liters of medium conditioned by the murine Neuro2A neuroblastoma cell line. This medium is concentrated 150–fold by an ultrafiltration step resulting into the precipitation of Astrostatine which is then collected by ultracentrifugation (100 000g, 30 minutes, 4°C). The pellet containing the Astrostatine activity is washed with tris buffer and extracted with tris buffer containing SDS, an anionic detergent. The solubilized proteins are then fractionated by a preparative electrophoresis on polyacrylamide gel using a Prep–Cell instrument. Pure Astrostatine is obtained with a molecular weight of 14 600 Da. It shows a moderate solubility in aquous solvent containing a zwitterionic detergent and exhibits an isoelectric point of about 4.9 to 5.0. Astrostatine is not glycosylated and its amino terminal extrimity is blocked. We are now performing microsequencing experiments after partial proteolysis of Astrostatine in order to asses its identity and to begin molecular cloning experiments. Astrostatine could represent an economical and power tool for neuronal cells to control and regulate the gliogenesis given the central role played by type 1 astrocytes. Moreover it could be implicated in the gliotic reaction and in the gliomatous transformation.

MORPHOLOGICAL CHARACTERIZATION OF THE ASTROCYTIC GAP JUNCTION COUPLING:

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Gap junctions (g.j.) apparently couple astrocytes to form the astrocytic network. Using stereological disector-counting the coupling pattern of astrocytes was quantitatively evaluated in adult rat visual cortex. First we estimated the probability to recognize gap junctions at various section angles in electron microscopical pictures. These morphometrical studies revealed that on average each astrocyte has 15,000 g.j. indicating a tremendous capacity for interaction between astrocytes. Isolated HRP-stained astrocytes also show g.j. between processes or lamellae of one stained cell. Hence, "**autocoupling**" (coupling between processes and lamellae of one cell) as well as "**heterocoupling**" (intercellular contacts) occur among astrocytes. Considering that on average 17,500 astrocytes exist per mm^3 of adult rat cortex, one g.j. is found in a tissue space of 3-4 μm^3. Since g.j. diameters range from 0.07-0.33 µm each astrocyte has a g.j. area of about 471 μm^2. According to stereological techniques (point/intercept-counting) the total contact area between astrocytic membranes is about 8,300 μm^2 per cell. Hence, gap junction area represents 6 % of the contact area between astrocytic processes and lamellae. These data suggest that the astrocytic network consists of subcellular units which are defined by gap junction coupling.

AN IN VITRO STUDY ON 1,3-DINITROBENZENE TOXICITY AT THE BLOOD-BRAIN BARRIER

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The blood-brain barrier (BBB) is able to exclude some potential toxins to the central nervous system, but certain agents that cause damage directly to the endothelium or to perivascular astrocytes can cause severe neurotoxicity associated with, and exarcebated by, BBB breakdown. *In vivo* 1,3-dinitrobenzene (DNB) causes glial swelling and vacuolation in certain brainstem nuclei associated with petechial haemorrhages and secondary neuronal death. We have studied changes associated with DNB poisoning in both astrocytes and brain capillary endothelial cells (BCEC) in primary culture. The threshold cytotoxic concentration for both astrocytes and BCEC was 1.0 mM over 1 day as assessed by lactate dehydrogenase leakage into the medium. Astrocytes were metabolically more active than BCEC in control dishes. Cumulative glucose consumption was 28±3 and 13±2 µmol/mg prot·day for astrocytes and BCEC, respectively. Lactate release into the medium was 37±5 µmol/mg prot·day for astrocytes and 14±2 µmol/mg prot·day for BCEC. Concentrations of 0.05 mM for astrocytes and 0.1 mM for BCEC and above caused a significant rise in glucose utilization, whilst increased lactate release was seen only at 0.1 mM in astrocytes and 0.5 mM in BCEC. NBT reduction, as an indirect method of oxygen radical production, was enhanced with 0.05 mM DNB in both cell types, although the response was significantly larger in astrocytes. Protective agents, such as catalase, glutathione or ascorbate, failed to reduce the rate of NBT reduction when cells where incubated with 0.5 mM DNB, while incubation with superoxide dismutase resulted in a significant 21±6% reduction. The percentage of oxidised glutathione rose from 8±1 to 23±4% when astrocytes were incubated with 0.5 mM DNB, and total glutathione content was depleted to 63±8% of control values 2 h after incubation with DNB. Cytotoxic concentrations of DNB decreased the autofluorescence (400-510 nm) of both cell types indicating a decrease in the intracellular NAD(P)H/NAD(P) ratio. These results show that *in vitro* models can give useful information about sites and mechanism of toxic action. A toxic effect of DNB both on astrocytes and on BCEC was observed, although astrocytes were somehow more sensitive to the action of DNB. A mechanism of action involving production of free radicals is suggested.

MODEL OF DYNAMIC pH-HOMEOSTASIS IN THE LEECH CNS

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We investigated the involvement of carbonic anhydrase and glial Na^+/HCO_3^--cotransport in the maintenance of extracellular pH-homeostasis during neuronal activity induced by repetitive electrical side nerve stimulation in the central nervous system (CNS) of the leech *Hirudo medicinalis*. The measurements were performed with double-barrelled pH-sensitive microelectrodes in the extracellular spaces (ECS), in neurones, and in neuropile glial cells of segmental ganglia.

Neuronal activity was accompanied by a small alkalinization followed by an acidification of the ECS, an acidification of neurones and an alkalinization of glial cells in CO_2/HCO_3^--buffered saline (1). The stimulus-evoked extracellular alkalinization is likely to be caused by the flux of acid-base-equivalents through transmitter-gated ion channels, which in parallel may lead to the acidification of neurones. The subsequent extrusion of acid via the Na^+/H^+-exchanger mainly mediates the recovery from the neuronal acidification and is responsible for the major part of the *extra*cellular acidification. Following inhibition of carbonic anhydrase by ethoxyzolamide (2µM), we observed a pronounced extracellular alkalinization during neuronal activity, whereas the intracellular pH-transients in both neurones and glial cells were uneffected. During application of DIDS (4,4-diisiothiocyanatostilbene-2,2′-disulphonic acid, 0.5 mM), a blocker of glial Na^+/HCO_3^--cotransport, the stimulus-evoked glial alkalinization was nearly completely inhibited. At the same time, the extracellular alkalinization was largely increased. We conclude that there is a significant amount of extracellular carbonic anhydrase activity in the leech CNS, which mediates the fast supply of acid-base-equivalents from the CO_2/HCO_3^--buffer system thereby reducing the activity-induced pH-changes in the ECS. Transport of bicarbonate into the glial cells following activation of Na^+/HCO_3^--cotransport leads to a further "muffling" (2) of the extracellular alkalinization.

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INDICATION OF TWO SUBPOPULATIONS OF CEREBELLAR ASTROGLIAL MITOCHONDRIA EXHIBITING GLUTAMATE DEHYDROGENASE

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For the ultrastructural localization of the glutamate dehydrogenase immunoreactivity (GDH-IR) in the rat cerebellar cortex we employed the post-embedding immunogold technique. The highest gold-particle densities were observed over astroglial mitochondria. To decompose a possible mixture distribution of mitochondrial subpopulations into normally distributed components we applied the program STARTVAL. The analysis was carried out for the whole pool of astroglial mitochondria of the cerebellar cortex layers including the white matter. In contrast with the fit of the normal distribution ($p = 0.025$), after fixation with glutaraldehyde and paraformaldehyde the mixture model was accepted ($p = 0.260$). The decomposition procedure identified two normally distributed components. Approximately 76 % of the astroglial mitochondria exhibited the subpopulation with lower GDH-IR (mean: 140 gold particle/µm²) and about 24 % the subpopulation with higher values (mean: 259 gold particles/µm²). The subpopulations exist independently on the morphological astrocyte types. Their numerical proportions in the different cerebellar cortex layers are equal in statistical sense.

PRELIMINARY CHARACTERIZATION OF A SCHWANN CELL GROWTH-INHIBITORY FACTOR FROM F7 CELLS

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Growth of Schwann cells prior to myelination may be curtailed in part by antiproliferative factors derived from the Schwann cell which overcome prevailing mitogenic signals in developing or regenerating peripheral nerves. Candidates for the autocrine antiproliferative activity include TGF-βs, a family of 13-18 kDa polypeptides[1] and the matrix protease, NAP[2]. Soluble Schwann cell Growth-Inhibitory Factor(s) (SGIF) has been identified in media from F7, a rat Schwann cell line which exhibits marked self-induced mitotic inhibition[3]. SGIF also promotes differentiation of the cells to a bipolar/stellate morphology. It is active in F7, primary rat Schwann cells and 33B schwannoma cells but not in WI38 fibroblasts. SGIF is heat- and acid-stable and adheres to the substratum. Both antiproliferative and differentiation-inducing activity bind to heparin-agarose and two peaks of activity are eluted at 0.2 and 0.8M NaCl. Coidentification of SGIF with other antiproliferative factors by further characterization is in progress. Since the F7 cell line both release and respond to SGIF, it seems unlikely that control of proliferation by this mechanism is lost on transformation.

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PRESENCE OF P- AND L-SELECTINS IN IMMORTALIZED RAT SCHWANN CELL LINE F7

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Contact and recognition by Schwann cells of axons and the perineurial basal lamina are required for the initiation of myelination in peripheral nerves. Myelin sheath formation also depends on the interaction of Schwann cell membrane glycosphingolipids (GSLs) with selectins or other transmembrane lectins. These interactions promote cell-substratum adhesion in Schwann cells. To investigate the role of GSL-lectin interactions in cell adhesion and myelin formation we have developed a normal rat diploid/hyperdiploid Schwann cell line (SCL4.1/F7) which can be induced to display a myelinating phenotype. P and L-selectins were localized to patches on the surface of F7 cells. MAb SK11 which competes with L-selectin ligands failed to prevent F7 cell-adhesion to poly-L-lysine. By comparison with 33B schwannoma cells which displayed near calcium-independent anchorage, F7 cells required both serum and calcium at more than 1mM for substratum adhesion. We conclude that GSL-L-selectin binding, a calcium-independent process, is not required for cell adhesion. By contrast, adhesion is strongly calcium-dependent and may involve P-selectin or other calcium-mediated recognition mechanisms.

PHORBOL ESTER MODULATES IODINATED OXYTOCIN ANTAGONIST BINDING ON CULTURED ASTROCYTES.

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Specific oxytocin (OT) receptors have been previously characterized on hypothalamic cultured astrocytes and intracellular Ca^{2+} mobilization consecutive to receptor activation by the agonist has been demonstrated. The involvement of phosphoinositide hydrolysis which generates IP_3 and diacylglygerol, an activator of protein kinases C (PKC), could be hypothetized. As stimulation of PKC appears to be an important event in transduction mechanisms and receptor regulation, the role of PKC on astroglial OT receptor regulation was studied on 12-day-old hypothalamic cultured astrocytes. Binding studies were performed on cells pretreated with a phorbol ester (phorbol-12 myristate, 13-acetate, PMA). Short-term exposure (up to 30 min) to PMA (10^{-7}M) or OT(10^{-9}M) alone was without effect on ligand binding whereas simultaneous addition of OT and PMA significantly decreased ligand binding and this effect was completely reversed by the PKC inhibitor, staurosporin. PMA long-term treatment is thought to desensitize PKC and in our system, 18 hours treatment with PMA or OT alone decreased ligand binding and simultaneous application of the drugs potentiated this effect which could not be reversed by staurosporin. In conclusion, the first step in agonist- and phorbol ester-mediated ligand binding inhibition, depends on PKC activation whereas down-regulation induced by long-term treatment is independent from PKC.

A NEW MEMBER OF THE GLUCURONOSYLTRANSFERASE- FAMILY IS PRESENT IN OLIGODENDROCYTES AND SCHWANN CELLS

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Myelinogenesis is a complex, developmentally regulated process involving coordinate expression of myelination-related proteins. The myelin forming cells are the oligodendrocytes in the central nervous system (CNS) and the Schwann cells in the peripheral nervous system (PNS). We differentially screened a rat postnatal day 16 (P16) spinal cord cDNA library with probes derived from normal (plus probe) and from myelin-free (minus probe) P16 spinal cord mRNAs. We succeded in the isolation of several novel cDNA clones whose corresponding mRNAs were expressed either selectively by oligodendrocytes or by oligodendrocytes and Schwann cells.

Here we describe one of the cDNA clones (tentatively denominated NS 2) whose mRNA is specifically expressed by oligodendrocytes and Schwann cells. The 3.5 kD transcript is expressed at highest level during myelination and at much lower levels in the adult as it is known for other myelin-specific mRNAs. Sequence analysis of the full length cDNA sequence showed a 50% amino acid identity to the rat liver UDP-glucuronosyltransferase family, involved in the detoxification system. The 541 amino acid open reading frame encodes a 62 kD protein with a putative signal peptide and two transmembrane domains. Whether this NS 2 protein is involved in a detoxification reaction or in glycosilation of proteins and lipids with glucuronic acid is under investigation.

SECOND MESSENGERS REGULATION OF INTERLEUKIN 6 PRODUCTION AND GENE EXPRESSION BY RAT CORTICAL ASTROCYTES

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Interleukin 6 (IL-6) is a pleiotropic cytokine whose role within the central nervous system (CNS) is still unclear. It is known that IL-6 production can be stimulated by inflammation-inducing agents such as IL-1β. Here we report data concerning the regulation of IL-6 production by agents acting via the activation of second messenger systems. VIP, a neurotransmitter with neurotropic properties, exerted via the interpolation of glial cells, stimulates in a concentration dependent manner IL-6 production by astrocytes. This stimulation was blocked by agents inhibiting protein kinase (PK) A activation, but not by PKC blockers, suggesting that this action of VIP is obtained via the activation of PKA. Both forskolin and cAMP congeners showed stimulatory effect on IL-6 production strengthening the observation that an increase of cAMP within the astrocyte causes IL-6 release.

Phorbol 13-myristate, 12-acetate (PMA), an activator of PKC elicited IL-6 production in a concentration related manner. This effect of PMA was antagonized by PKC blockers and by PKC desensitization, strongly suggesting that it is exerted via the activation of PKC. In addition PMA strongly synergized with IL-1β induced IL-6 production and gene expression, suggesting that inflammatory and PKC mediated stimuli can interact inducing an overproduction of IL-6 production by cortical astrocytes.

STIMULATION OF ASTROGLIAL ARGININE TRANSPORT BY BACTERIAL LIPOPOLYSACCHARIDE

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Astrocytes express an isoform of NO synthase which is induced by lipopolysaccharide (LPS; Simmons and Murphy,1992). L-arginine as the substrate of the enzyme is taken up into astrocytes with the help of transport system y^+. This process may be an important control point in the production of the messenger molecule NO.

Rate of uptake of L-arginine into astroglia-rich primary cultures derived from the brains of newborn mice or rats was increased at least 1.7-fold when the cells had been preincubated with LPS. Stimulation of uptake started 6 hours after begin of the preincubation and reached its maximum value after 14 hours. Half-maximal stimulation of uptake was achieved with LPS at a concentration of 0.5-1 µg/ml. No decrease in K_M was detected when the uptake was measured as a function of the concentration of extracellular arginine after 16 hours of preincubation with LPS. When the cells were preincubated with LPS in the presence of cycloheximide, no stimulation of arginine uptake could be detected. The results allow the conclusion that LPS stimulates the synthesis of y^+ transporter protein and its insertion into the astroglial plasma membrane. Obviously, a fine tuning of arginine consumption by NO synthase and arginine supply through transport is operating in astrocytes.

GLUCONEOGENESIS IN ASTROGLIAL CELLS

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Astroglia-rich primary cultures derived from newborn rat and mouse brains contain glycogen, which is degraded during lack of glucose or under the influence of hormones and re-synthesized on refeeding with glucose (1). During the resynthesis of glycogen, label from radioactive lactate, is incorporated into the glucosyl- residues of glycogen, a process, which can be considered as a variant of gluconeogenesis. Also aspartate, alanine, and glutamate can serve as precursors of these glucosyl-residues. The incorporation of radioactive label was inhibited by 3-mercaptopicolinic acid, an inhibitor of phosphoenolpyruvate carboxykinase (PEPCK), beside fructose-1.6-bisphosphatase (FBPase) one of the key gluconeogenic enzymes. The specific activities of PEPCK and FBPase in astroglia-rich primary cultures amount to 5 to 10 % that of rat hepatocyte primary cultures. In contrast to hepatocytes the astroglia cells express only the mitochondrial and not the cytosolic isoenzyme of PEPCK. Dibutyryl cyclicAMP and dexamethasone, inducers of PEPCK in hepatocytes, do not influence the activity of this enzyme in astroglia-rich primary cultures.

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PATTERN OF SOMATOSTATIN BINDING SITES ON CULTIVATED GLIAL TUMOURS AND GLIAL CELL LINES

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Rat astrocytes *in situ* and *in vitro* are endowed with functionally relevant binding sites for somatostatin as has been shown for its complex with colloidal gold[1]. When human gliomas are screened *in situ* for their affinity for somatostatin by scintigraphy using the ^{111}indium-labelled somatostatin agonist DTPA-octreotide as ligand, some of them are negative. In contrast, when parts of gliomas obtained during operations are taken in culture and analyzed for somatostatin binding sites by use of a somatostatin-gold conjugate, all cultivated tumours contain, besides unlabelled cells, a considerable number of heavily somatostatin-gold labelled cells. They prove to be glia cells due to immunohistochemical criteria. The distribution pattern of bindings sites differs among the tumours and changes with the number of subcultivations. Corresponding findings are achieved with C6-glioma cells: after the 2nd or 3rd subcultivation, many of them bind somatostatin-gold in nanomolar concentrations. However, always a part of the cells is completely devoid of binding sites for somatostatin. The binding of somatostatin-gold can be competitively inhibited by surplus of unlabelled peptide. Furthermore, three established human glioblastoma cell lines are enclosed in this study.

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PROGESTERONE : AN AUTOCRINE FACTOR WITHIN THE PERIPHERAL NERVOUS SYSTEM

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The neurosteroid progesterone (PROG) is synthesized from pregnenolone by glial cells in the brain, where it stimulates the synthesis of myelin specific proteins (poster I. Jung-Testas et al.). PROG is also produced by Schwann cells of the peripheral nervous system. Although pure cultures of Schwann cells, isolated from neonatal rat sciatic nerves, do not convert [^{3}H]pregnenolone to PROG, cocultures of Schwann cells and dorsal root ganglion (DRG) neurons produce PROG and its reduced metabolites pregnanedione and allopregnanolone. The production of allopregnanolone is increased five-fold when cultures are placed in a medium that activates myelination.

Schwann cells are a source of PROG. Indeed, when neurons are removed from the cultures by cutting out the ganglion explants, the remaining Schwann cells convert pregnenolone to PROG, even shortly after trypsination. In Schwann cells, neurons induce the enzyme which converts pregnenolone to PROG, 3ß-hydroxysteroid dehydrogenase, and also the receptor of PROG (PR). The PR mRNA was detected by RT-PCR in cocultures of Schwann cells and DRG neurons after removing the ganglia, but not in Schwann cells isolated from sciatic nerves. These observations strongly suggest that the synthesis of PROG and the expression of its receptor in Schwann cells are induced by neuronal factors, either secreted or membrane bound. Thus, the functional activities of Schwann cells, such as myelination (poster H. Koenig et al.), would be regulated by PROG through autocrine actions.

ANALYSIS OF AMPA/KAINATE RECEPTORS IN HIPPOCAMPAL GLIAL CELLS

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It is well known that glial cells express different types of ligand-gated ion channels. Recently, it was demonstrated that glutamate and kainate activated receptor channels and simultaneously blocked voltage-gated K^+ channels in hippocampal glial cells in situ. It was speculated that the channel was of the high-affinity kainate subtype of ionotropic glutamate receptors since the current-to voltage relationship was linear (having a reversal potential of about 0 mV) and since the receptor pore possessed a significant Ca^{2+} permeability.

In the present study we further characterized this putative glial kainate receptor. Since voltage clamp control is imperfect in situ and diffusion barriers make a quantitative analysis of receptor currents impossible, the investigations were extended to cells acutely isolated from tissue slices prior to voltage clamp analysis. We focused on cells expressing the same typical pattern of voltage activated K^+ and Na^+ currents which previously was found in 'complex' hippocampal glial cells in situ. A fast concentration clamp technique was developed which enabled a complete exchange of extracellular solutions within 3-4 ms. Thus, it was possible to characterize fast transient receptor currents with high time resolution. We show that application of glutamate and AMPA induced very fast activating and rapidly desensitizing receptor currents in suspended 'complex' glial cells. In contrast, desensitization of the kainate receptor was considerably slower and in most cases followed a biphasic time course. The corresponding dose-response curve revealed that maximum current amplitudes were induced at concentrations of about 1 mM while application of kainate concentrations below 50 µM did not activate any responses. This study unequivocally proves that glial cells of the juvenile hippocampus express AMPA/kainate receptors. The data refute the argument that the kainate sensitivity of glial cells in slice preparations resulted from the release of unknown substances which then indirectly affected the cells recorded from.

GFAP FLUCTUATIONS OVER THE 24H PERIOD IN THE CIRCADIAN CLOCK OF RODENTS.

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Immunoreactivity for GFAP is particularly dense in the suprachiasmatic nucleus (SCN) of rodents. The SCN is the site of the main endogenous pacemaker generating a variety of circadian rhythms. The SCN itself exhibits clear rhythms in glucose consumption, electrical activity and peptide expression. We recently demonstrated a circadian rhythm in GFAP immunoreactivity (Ir) distribution in the hamster SCN. The present study extended our initial observations over more numerous circadian times (CT) using both immuno-histochemistry and western blotting methods. In order to verify whether this phenomenon is of general value, parallel studies were performed on rat. Male animals were raised in photoperiod LD 12:12 (12h light/12h dark). To assess internal clock time, locomotor activity was recorded (activity onset = CT12). Animals were taken at various CTs (CT02, 06, 10, 14, 18, 22). Immunohistochemistry was performed on fixed brain sections and Western blotting on SCN micropunches. In hamster, daily fluctuations in GFAP immunoreactivity distribution (Ir) and GFAP content were characterized, at the beginning of the night, by a change in GFAP-Ir from a network pattern to isolated cells and by a decrease in protein level. GFAP-Ir rhythm was also present after 8 cycles in DD. These rhythms exhibited a profile similar to that of glucose consumption with a marked trough at CT14. In rat, the first data also indicate a nycthemeral variation. It has to be pointed out that under physiological conditions, these unexpected modifications in GFAP can be regarded as a new index of the rhythmic activity of the clock.
Since glutamate content exhibits a circadian rhythm in the SCN, glutamine synthetase (GS) could then constitute an additional index of circadian activity of the clock. GS study has been undertaken.

CHARACTERIZATION OF A DOPAMINE SURVIVAL FACTOR FROM VENTRAL MESENCEPHALIC TYPE 1 ASTROCYTES

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Previous work from our laboratory has shown that ventral mesencephalic (VM) Type 1 (T1) astrocytes selectively enhance dopamine (DA) neuron survival in vitro. Moreover, a heat and proteolysis-sensitive factor in T1 astrocyte-conditioned medium (CM) reproduces the survival effect, suggesting that a soluble protein is responsible. In parallel with protein purification studies, we have used dissociated neuronal cultures of embryonic rat VM to compare the biological activity of CM with that of several previously identified neurotrophic molecules. Although basic fibroblast growth factor (bFGF), like CM, increases the survival of DA neurons in vitro, it requires the presence of glia to mediate survival, whereas CM acts directly on neurons. Members of the neurotrophin gene family, especially brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), reportedly have trophic effects on DA neurons in culture. CM increased DA neuron survival to a far greater degree than either BDNF or NT-3, although both neurotrophins are expressed in VM T1 astrocytes. These results alone, however, cannot discount the possible involvement of members of the neurotrophin gene family in the CM survival effect. Treatment of neuronal cultures with the tyrosine kinase inhibitor K252a, which blocks the trk receptor-mediated effects of neurotrophins, failed to inhibit the effect of CM on VM DA neurons. These data suggest that the CM factor is not a member of the neurotrophin gene family, either previously identified or novel. We have recently shown that VM T1 astrocytes express glial cell-line derived neurotrophic factor (GDNF; Schaar et al, 1993), a molecule with selective effect on DA neurons in vitro. We are presently investigating whether this molecule is involved in the CM survival effect.

CHRONO-TYPICAL PATTERN OF INJURY-INDUCED ACTIVATION OF MICROGLIAL CELLS IN THE RAT RETINA AND OPTIC NERVE

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The role of microglia in brain, as an immunologically privileged site, which is shielded from the immune system by the blood brain barrier, becomes increasingly apparent in injury-induced lesions.
In the present study we examined activated and phagocytosing microglial cells at different days after crush of the optic nerve and analysed their numbers in the retina and in the nerve itself. To detect the microglia in the retina, we used the method of phagocytosis-dependent labelling of fluorescently filled neurons and for comparison the NDPase-method. The experiments were performed in in albino rats from the SPRD-strain and in RCS-rats with hereditary photoreceptor dystrophy. To determine the numbers of microglia in the optic nerve, cryostat sections were incubated with antibodies MUC 101 and 102. We found an increasing number of phagocytosing microglial cells in the retina between day 3 and 12 after crush and a significant increase of activated microglia in the crushed optic nerve compared to the uncrushed control. Between day 6 and 12 after crush no significant difference was found in the number of activated microglia identified by their typical ramified form in the optic nerve and detected by MUC 101/102. At the injury-sites as well as in the destroyed layer of photoreceptors in the retina of the RCS-rat the shape of microglia changed from the ramified into a rounded, swollen form.
The results show that immunohistochemical detection of activated microglial cells precedes phagocytosis-dependent staining. Both properties emphasize the significance of microglial cells in injury-induced decay of neurons.

THE ROLE OF PKC IN DIFFERENTIATION AND PROLIFERATION SIGNALLING IN ASTROCYTES FROM CHICK EMBRYO CULTURES

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Astrocytes are important, integral parts of CNS organization, development and normal function. Additionally they carry out specific roles during diseases (genetic, viral or trauma). Much of these functions are regulated by G–protein linked membrane receptors, coupled to protein kinase C activation (after diacylglycerol generation). We have previously shown that in astrocytic cultures from day–15–chick embryo cerebral hemispheres (E15CH) the major PKC isoform is the DAG and Ca^{2+}–dependent α/β (Mangoura et al., J.Neurosci.Res.,35:488,1993). This isoform is expressed in all primitive neural cells and remains associated with the glia cell lineage, whereas PKC–ϵ appears as strictly neurons associated. In this study we investigated the protein kinase C signal transduction pathway as activated either directly by phorbol ester (TPA), or after stimulation of the G_{PLC}–coupled muscarinic receptor by the homologous agonist carbachol, in E15CH cultures. Both TPA and carbachol elicited a rapid translocation of PKC activity within min after treatment. Carbachol stimulated IP hydrolysis in a time and dose dependent manner, achieving a 4–fold maximum. Pirenzepine, but not gallamine, attenuated carbachol–induced IP hydrolysis, arguing for a M_1 receptor subtype. However, phospholipase D (PLD) activation was mostly dependent on the M_3 receptor subtype. TPA did activate PLD in a time and dose dependent manner and PLD activity was much higher than that found in neurons. Furthermore, while short term TPA treatment resulted in phosphorylation of several phosphoproteins (vimentin, GFAP) in the glia cytoskeletons, long term TPA treatment changed overall the astrocyte phenotype as assessed by decreased in GFAP expression and glutamine synthetase activity, increases in vimentin expression, and cell shape and proliferation. Treatment with staurosporin, a PKC inhibitor, resulted in partial inhibition of the TPA effects. These data suggest that PKC is an important effector of biological events in glial cells for their differentiation and function.

ADULT RAT OLFACTORY GLIAL CELL LINES SUPPORT NEURITE REGROWTH FROM CO-CULTURED ADULT RAT CNS NEURONS.

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The survival and regeneration of axons following injury to the nervous system is thought to depend on neuronal interactions with local glial cells. The primary sensory neurons of the olfactory system are generated throughout life from a neuroepithelium and their axons grow into the central nervous system (CNS) to reinnervate targets in the olfactory bulb. Axons are accompanied along the nerve and into the CNS by olfactory glial /ensheathing cells, which are a unique cell type with properties of both peripheral Schwann cells and astrocytes. We have isolated cells from the intracranial portions of olfactory nerves of adult rats and characterised the phenotype of cells in primary culture and derived cell lines. Five independently initiated lines have been isolated as a result of the unusually rapid proliferation of olfactory cells during the first few passages. The proliferative cells retained some features of ensheathing cells but lost others. Modulation of culture conditions by several different means induced re-expression of the differentiated phenotype. In co-culture with neurons isolated from adult rat retina, olfactory glia supported the survival and regrowth of extensive neurites from retinal ganglion cells (RGC). This support was comparable or superior to that seen with neonatal astrocytes or Schwann cells in parallel experiments. However, in contrast to our findings with Schwann cells, olfactory glia conditioned medium had little effect on either survival or neurite outgrowth from RGC. In co-culture, we consistently scored 30-fold more Thy-1 - positive, β tubulin III - positive RGC with neurites than on a polylysine surface, with or without olfactory glia conditioned medium. Isolated adult RGC show no preference for EHS laminin over polylysine as a substrate (Kabir and Wigley, unpublished data). Thus we are now investigating the potential importance of neuronal cell surface adhesive interactions with cell surface or alternative matrix molecules of olfactory glial origin.

HUMAN ASTROGLIAL CELLS EXPRESS GRANULOCYTE COLONY-STIMULATING FACTOR *IN VIVO*

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Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic cytokine that regulates the differentiation of myeloid progenitors and the function of mature neutrophils. It is known to be produced *in vitro* by monocytes/ macrophages, mesothelial cells, fibroblasts and endothelial cells after appropriate induction by inflammatory mediators like IL-1 and TNF. Normal as well as tumorous glial cells can also be induced to produce CSFs *in vitro*. However, little is known yet about the *in vivo* expression of G-CSF as a mediator in inflammation and malignancy within the human central nervous system. The aim of the present study was to investigate by immunostaining the expression of the G-CSF protein within non-tumorous and tumorous glial tissues, and primitive neuroectodermal tumors. Using the murine monoclonal anti-G-CSF TM 82/60 antibody, we found high G-CSF expression in astrocytoma WHO grades I and II and reactive brain tissue, low expression in astrocytoma WHO grade III, and none in glioblastoma, oligodendroglioma WHO grades II and III, and medulloblastoma. In consecutive sections of the tissue samples G-CSF protein was localized in GFAP positive glial cells, but not in macrophages/microglial cells, which expressed HLA-DR, detected by the antibody CR3/43. Computer-assisted microdensitometric evaluation of the intensity of immunostaining for G-CSF and statistic analysis of the data revealed significant differences between the studied diagnostic entities ($p < 0.0001$). We conclude, that *in vivo* expression of G-CSF is a characteristic of reactive as well as tumorous astrocytes, with the latter loosing this feature at higher degrees of dedifferentiation.

ARACHIDONIC ACID MEDIATES CYTOTOXIC GLIAL SWELLING

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Introduction: Cerebral ischemia and trauma are associated with massive release of arachidonic acid (AA) in brain tissue. The fatty acid and its metabolites mediate pathophysiological processes, resulting in brain edema and irreversible tissue damage. Presently, mechanisms of AA-induced swelling of glial cells were investigated in vitro.

Methods: C6 glioma cell- or primary cultured astrocyte-suspensions were added with AA at 0.01 mM to 1.0 mM. The resulting cell volume response was assessed by flow cytometry. Additional experiments at 0.1 mM AA were conducted in the presence of BW 755C for inhibition of cyclo- and lipoxygenase products, superoxide dismutase (SOD), or an aminosteroid (U-74389F) for scavenging of lipoperoxides. Further, inhibition of Na^+/K^+-ATPase by ouabain in AA-induced cell swelling was studied as well as replacement of Na^+-ions in the medium against choline chloride.

Results: AA led to a dose-dependent swelling of C6 glioma cells or of astrocytes, at concentrations as low as 0.01 mM ($p<0.01$). AA at 0.01 mM led to an increase of the glial cell volume to 105% of control, 0.01 mM to a cell volume of 110%, while 1.0 mM to 120%. Glial swelling from AA was not inhibited by BW 755C, whereas it was reduced by SOD ($p<0.05$), or almost completely prevented by the aminosteroid ($p<0.01$), respectively. Addition of ouabain, or replacement of Na^+-ions by choline chloride in the medium was also associated with complete abolishment of cell swelling from AA ($p<0.01$).

Conclusions: The current findings confirm powerful swelling-inducing properties of AA at concentrations found in cerebral tissue in-vivo, as e.g. ischemia. AA-induced cell swelling cannot be attributed to the formation of prostaglandines or leukotrienes, while free O_2-radicals and lipoperoxides seem to be involved. Inhibition of AA-induced cell swelling by ouabain or by suspension of the cells in a Na^+-free medium demonstrates the significance of the extra/intracellular Na^+-gradient and Na^+-net influx in the swelling process. The present findings provide novel information on mechanisms underlying AA-induced glial swelling as a basis for the development of therapeutical methods of inhibition.

AXONAL SPROUTING THROUGH A LAMININ-FREE ASTROGLIOTIC REGION OF THE TRANSECTED FORNIX TRACT

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Lesion-induced regenerative sprouting of CNS axons is accompanied by structural and metabolic changes of astrocytes. In order to evaluate the effects of the astrocytic changes on axonal regeneration, we investigated the spatio-temporal relationship of astrogliosis formation, laminin(LN)-expression and axonal sprouting in the postcommissural fornix of the adult rat.

The fornix tract was stereotactically transected and after 3d up to 28m post-lesion(PL) astrocytes, laminin and axons were visualized using mono- and polyclonal antibodies against glial fibrillary acidic protein (GFAP), S-100, vimentin, EHS sarcoma-LN and all neurofilament subunits.

The major findings of this study were: (1) the appearance of a perilesional area with a transient lack of astrocytes and axons, (2) the reappearance of reactive astrocytes followed by the ingrowth of regenerating fibers and finally an increase in LN-immunoreactivity, (3) the absence of injury-induced LN-expression in reactive astrocytes and (4) the formation and long-lasting (at least 28 m) persistence of a dense plexus of LN-immunopositive blood vessels first at the site of transection and later (from PL4w onwards) in the proximal and distal stumps.

These data indicate that (1) reactive astrocytes reorganize around the lesion site, (2) astrogliosis is permeable for regenerating axons and (3) injury-induced axonal sprouting in the transected fornix tract may be mediated by LN-independent mechanisms.

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RE-EXPRESSION OF THE CELL ADHESION MOLECULE N-CADHERIN-A NEW INDICATOR FOR PLASTICITY IN ADULT CANARY BRAIN ?

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One of the best known functional systems of the vertebrate CNS is the vocal control feedback-loop of canaries and zebra finches which mediates song production. In adult male canaries, neuroneogenesis, a phenomenon characteristic of development, continues in adult life in a seasonal rhythm. In autumn, cells are born in the walls of the lateral ventricle. These newly generated neuroblasts migrate and differentiate throughout the adult telencephalon (1). Radial glial cells which may be essential for the migration of young neurons, also persist in adult canary brain. The remodeling of neuronal substrates correlates with vocal learning in adulthood. Here we show that, during seasonally dependent rejuvenation, there is a re-expression of N-cadherin by radial glial cells in the adult male canary brain. N-cadherin is a Ca^{2+}-dependent cell adhesion molecule that has been shown to be expressed by radial glia and by particular developing nuclei and fiber tracts of the embryonic chicken brain (2). This molecule may be involved in the formation and segregation of functional systems in the vertebrate CNS. Our results suggest that N-cadherin plays a role in the reconstruction of neuronal and behavioral resources of the canary.

1) Alvarez-Buylla, A. 1992, Exp. Neurol., 115, 110-114;
2) Redies, C. et al 1993, J. Comp. Neurol., 333, 398-416

FORMATION OF A BASAL LAMINA IN COCULTURES OF MENINGEAL CELLS AND ASTROCYTES

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The central nervous system (CNS) is bordered by the glial limiting membrane which consists of glial endfeet covered by a basement membrane and separates the pia mater from neural tissue. The events leading to the formation of the glial limiting membrane are largely unknown. However, a similar structure, the accessory glial limiting membrane, is formed in the course of cicatrization after penetrating injuries to the CNS. The formation of the accessory glial limiting membrane seems to depend on the invasion of meningeal cells into the wound. Accordingly, in immature animals no astroglial scar forms due to the lack of a meningeal invasion. Thus, obviously astro-meningeal interactions are important for the formation of the glial limiting membrane. In the present study the interactions of astrocytes and meningeal cells were investigated in vitro to define more closely the conditions leading to the formation of a glial limiting membrane including the overlying basement membrane. In cocultures of meningeal cells and astrocytes of two day old rats following observations were made: (i) morphological transformation of epithelioid into stellate astrocytes; (ii) generation of single colonies of proliferating epithelioid astrocytes; (iii) the area around these colonies becomes devoid of meningeal cells, which seem to form a circular border around the astroglial islands; (iv) from the glial colonies long thin glial processes grow towards the surrounding meningeal cells, stopping at the site of contact; (v) in the contact zone between meningeal cells and astrocytes irregular shaped deposits of electron dense material resembling a basal lamina are seen.

ISOPROTERENOL INDUCES CHANGES IN THE NUMBER OF SYNAPTOID CONTACTS BETWEEN PITUICYTES AND NERVE TERMINALS IN THE RAT NEURAL LOBE

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Pituicytes exhibit a remarkable degree of plasticity in response to increased demand for neurohypophysial hormone release. The release of oxytocin (OXT) and vasopressin (AVP) from the neural lobe is regulated by a variety of substances, such as opioid peptides and probably also by OXT and AVP themselves. That release is not restricted to the nerve endings that abut the capillaries, but also occurs deeper in the neural lobe. These sites are most probably the synaptoid contacts. OXT and AVP-containing terminals made synaptoid contacts with pituicytes. Isolated neural lobes were incubated "in vitro" with isoproterenol and processed for electron microscopy. The number of synaptoid contacts of nerve terminals with pituicytes changed in response to beta adrenergic stimulation in "in vitro" neural lobes, as compared to controls. The different number of synaptoid contacts observed in the stimulated neural lobes is concomitant with changes in the morphology of pituicytes. It might be suggested a possible role of the pituicyte in the regulation of the release of OXT and AVP from nerve terminals.

MICROGLIAL CONTAMINANTS AS THE SOURCE OF IL-1 IN ASTROCYTE CULTURES

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IL-1 is a key regulator of the cytokine network within the nervous system. It stimulates its own production by microglial cells as well as the production of a range of cytokines by astrocytes like TNFα, TGFβ-1, IL-6 or GM-CSF. While there is no consensus on the sources of IL-1 in the CNS it is often assumed that astrocytes as well as microglia can be stimulated to produce IL-1. Since astrocyte cultures in previous studies were potentially contaminated by low but significant proportions of microglial cells we established microglia free astrocyte cultures to study the question of the cellular source of IL-1 in glial cultures. From confluent glial cultures of cortices of 2 day old BALB/c mice the bulk of microglial cells was removed by the usual shaking procedure. Residual microglia was quantitatively eliminated by three cytolytic cycles with Mac-1 antibodies and rabbit complement. Following that procedure less than one microglial cell could be detected per 10^6 astrocytes and astrocyte-oligodentrocyte precursors upon microscopic inspection of the Mac-1 and LDL receptor immunofluorescence labeled cultures. Supernatants of microglia free astrocyte cultures stimulated for three days with either E coli LPS (40ng - 10µg/ml), mIFN-γ (100 U/ml) or mTNFα (400-2000 U/ml) did not contain any IL-1 activity as assessed with the IL-1 specific T helper cell line D10.G4.1. In contrast supernatants of pure microglial cultures or astroglial cultures spiked with a proportion of 2.5‰ microglial cells contained IL-1 activity - 5ng mIL-1 eqivalents per 10^6 microglial cells and ml (LPS stimulation) -.
Conclusion: Cultured pure astrocytes in contrast to microglia do not release IL-1 upon LPS, IFNγ or TNFα stimulation. Earlier reports of IL-1 production and release by cultured astrocytes are most likely due to minute contaminants of microglia. The assumption that astroglia is a source of interstitial IL-1 in the CNS should be reevaluated.

ROLE OF GLIA IN EXTRACELLULAR K^+, pH AND VOLUME HOMEOSTASIS

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Neuronal activity is accompanied by long-term ionic and volume changes in the cellular microenvironment. Glial cells play an important role in K^+, pH and volume homeostasis, while their electrophysiological properties are related to the extracellular ionic and volume changes. Activity-related extracellular K^+, pH and diffusion parameters were studied by means of ion-selective microelectrodes in spinal cord and cortex of 1-90-day-old rats (P1-P90). Some rats were X-irradiated at P1 to block gliogenesis. At P2-P8 but later only in X-irradiated rats stimulation of an afferent input evoked an alkaline shift, K^+ ceiling level was elevated and extracellular space volume (ECV) occupied 35-45% of the total tissue volume. In normally developing rats the K^+ ceiling level began to decrease at P10-P12 and the stimulation evoked alkaline shift was followed by an acid shift. The acid shifts were blocked and alkaline shifts enhanced by acetazolamide, Ba^{2+}, amiloride, SITS and in bicarbonate free solution. Alkaline shifts were blocked by Ca^{2+}-channel blockers, picrotoxin and glutamate receptors antagonists. X-irradiation, anoxia and demyelinating diseases (EAE) resulted in changes in ionic homeostasis, ECV and in glial cells morphological and functional properties.

OLIGODENDROGLIA-MYELIN COMPLEX IN RATS SUBJECTED TO EXPERIMENTAL CLINICAL DEATH

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In experimental model of clinical death induced in rats using the method of Korpachev et all (1982) the oligodendroglia-myelin complex was evaluated using the morphometric and chemical methods of investigations. The results documented acute oedema of oligodendroglia nuclei in the early period, and degenerative changes later on. The studies of myelinated fibers seems to testify some differences in sensitivity according to the axon and myelin diameter on the influence of ischemia. The most pronounced changes in myelin lipids involved a marked increase in cholesteryl esters and lysophosphatidylcholine content. The late changes of myelinated nervous fibers in the postresuscitation syndrome seems to be the secondary effect of autoimmune dependent neuronal destruction, whereas the alterations of oligodendroglia only partially contribute to the myelin lesion. The result indicate also that ischemia induces a lesion of myelin membranes but does not provoke the reaction of lymphocytes and macrophages, which by production of myelinolytic factors, evoked demyelination.

CORRELATION BETWEEN HISTOPATHOLOGY AND MAGNETIC RESONANCE IMAGES OF A SPINAL DEMYELINATING LESION

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In order to compare the histopathology and MRI appearance of demyelinating lesions, focal areas of demyelination were created in the dorsal columns of the thoracolumbar spinal cord of cats by injecting 2µl of 0.1% ethidium bromide. Such lesions resolve by Schwann cell remyelination (Blakemore *Neuropathol Appl Neurobiol* **8**, 365, 1982).

Animals were imaged under general anaesthesia using a 2T, 31cm bore MRI system. 2mm thick slices, 7.56cm field of view, 256^2 matrix images were produced using a purpose built surface coil. Positioning of target images from scout scans using skeletal landmarks allowed spatially accurate correlation with subsequent histological examinations.

Diffusion weighted images aligned parallel to the longitudinal axis of the spinal cord identify areas of gliosis containing remyelinated axons as regions of increased signal intensity while areas of Schwann cell remyelination did not differ in appearance from normal white matter.

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In our previous studies we demonstrated that central neurons were able to produce the cytokine TNFα at both the mRNA and protein level after a hippocampal injury in the adult mouse brain (Tchélingérian et al. Neuron 10,213-224, 1993; Neuroreport in press). It was therefore important to identify the neural cells expressing the specific receptors. Indeed, TNFα may bind to two types of receptors, the p55 (typeI) TNFα receptor and the p75 (typeII) TNFα receptor which is highly homologous in its extracellular domain to the p75 NGF receptor. In this study we have used a non-radioactive in situ hybridization method to detect the gene expression of TNFα receptors (mRNAs) in normal and injured adult mouse brains. Both probes were antisense oligonucleotides of 30 mer labeled with digoxigenin. They were designed to avoid sequence homology between each probe and with the NGF p75 receptor. In the normal brain both receptor mRNAs were expressed in neurons of the hippocampus and other neurons in different brain areas. The strongest expression was for the p75 TNFα receptor. A non-neuronal expression was observed in meninges and white matter tracts with both receptors. After injury, mRNAs for both receptors were altered probably in relation with the massive expression of TNFα which is induced after brain injury. We hypothesize that the cytokine TNFα which is produced by the neurons after brain injury, can interact in an autocrine and paracrine fashion with its specific receptors which are also present on central neurons.

TRANSIENT EXPRESSION OF HIGHLY SIALYLATED NEURAL CELL ADHESION MOLECULE (PSA-NCAM) IN NEURONS AND ASTROCYTES OF THE ADULT RAT SPINAL CORD IN RESPONSE TO DORSAL RHIZOTOMY.

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PSA-N-CAM is expressed both in the developing nervous system and in certain adult neuronal systems and we have thus seen that PSA-NCAM immunoreactivity (IR) continues to be visible in the normal rat spinal cord, restricted to the grey matter of laminae I, II and X (Bonfanti et al 1992, Neuroscience 49,419). We here used light microscopic immunohistochemistry and an antibody specific for PSA-NCAM to examine this IR in the spinal cord of adult rats that underwent unilateral cervical (C4-C8) dorsal rhizotomy. There were no differences in PSA-NCAM-IR in the superficial layers of the dorsal horn in normal and lesioned animals, even 30 days postlesion. On the other hand, as early as 24 hours after rhizotomy, PSA-NCAM-IR occured in neuronal processes and somata in the dorsal horn adjacent to the lesion and throughout the grey matter on the controlateral side. This neuronal reaction was strongest 4 days postlesion and disappeared by 8 days. Four days postlesion, PSA-NCAM-IR was also visible in activated astrocytes; GFAP staining of adjacent sections confirmed that gliosis had begun at this time. The astrocytic reaction was particularly strong 8-12 days postlesion; it then progressively diminished but immunopositive astrocytes were still visible 40 days later. PSA-positive astrocytes were present in grey and white matter. They were particularly numerous in the dorsal horn adjacent to the lesion and in the dorsal columns; some were visible in the nucleus cuneatus. The immunolabel was punctate on the surface of the neuronal profiles while it filled the cytoplasm of astrocytes. Our observations show that PSA-NCAM-IR normally visible in the rat spinal cord is not carried by primary sensory afferents. In addition, they reveal that primary deafferentation results in a transient expression of PSA-NCAM by subsets of neurons and astrocytes that do not express it under normal conditions, an expression which shows a spatiotemporal pattern specific to each type of cell.

MYELINATION PROPERTIES OF A RAT OLIGODENDROCYTE CELL LINE AFTER TRANSPLANTATION INTO NORMAL AND MYELIN DEFICIENT HOSTS

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Transplantation of oligodendrocytes into the central nervous system is a promising approach to achieve remyelination to cure or at least improve neurodegenerative and especially demyelinating diseases. The cell line (CG4) used in this study has been established from primary glial cell cultures of newborn rat cerebral cortex (Louis et al., J.Neurosci.Res. 31, 193, 1992). Bipolar CG4 cells were stably transfected with the LacZ gene and the neomycin resistance gene and selected in the presence of G418. For grafting experiments, either into newborn rat brain or spinal cord of newborn myelin deficient rats (md), only cell populations containing 70-90% blue cells (as determined by X gal staining or immunoperoxidase labeling) were used. These cultures were indistinguishable from non-transfected cells with respect to their potential to proliferate in the presence of growth factors or to differentiate into oligodendrocytes (GalC and MBP positive) after growth factor withdrawal. Oligodendrocyte progenitors were carried through 40-48 passages and grafted into the cortical region close to the radiatio corporis callosi (50.000 cells in 1 ul). The cells migrated from the site of injection into the forming white matter where they nested with host oligodendrocytes into myelinating bundles. As well transfected as non-transfected CG4 cells were injected into the T13/L1 region of the spinal cord of normal or md rats. Two weeks after injection extensive myelination was found in the dorsal columns and the myelinated area spanned up to 12 mm rostral and caudal of the injection site. Our finding that an oligodendrocyte cell line, even when genetically modified, is able to survive, to migrate and to differentiate into myelin-forming cells upon transplantation into normal and hypomyelinated tissue significantly extends previous demonstrations of remyelination by implanted cells.

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MAP kinase cascade in astrocytes

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The multiplication and the differentiation of astroglial cells are controlled by growth factors (FGFs, IGF1, EGF, PDGF) and by hormones (endothelin, noradrenaline...). It has been shown in a number of cell types that growth factors receptors with tyrosine kinase activity transduce signal through small G proteins and serine/threonine kinases including the Mitogen-Activated-Protein Kinase (MAP-K). MAP-K appears to belong to a cascade of kinases involving MAP-K activator (MEK) and c-Raf. On another side, hormones which stimulate multiplication and differentiated functions, transduce signal via receptors coupled to large G proteins and protein kinases A and C.

In cultured rat astroglial cells, MAP-K is activated by various growth factors : FGFb, FGFa, EGF, PDGF, IGF1. We have observed the activation of MEK and the presence of cRaf. The kinetics of MAP-K activation are different for each factor. So, FGFb induces a rapid activation of MAP-K (about 10 min) followed by a sustained phase lasting at least 12 h. IGF1 produces only a rapid transient peak of activation at about 20 min. We have shown also that hormones acting through G proteins (endothelin, carbachol, TSH) activate MAP-K by pathways which remain unidentified. So we have looked for an effect of TSH since the presence of TSH receptors and the stimulation of type II iodothyronine 5'-deiodinase by TSH in cultured rat astroglial cells have been recently reported (Saunier et al., 1993, Eur. J. Biochem., 218, 1091-1094).

In astroglial cells, MAP-kinase cascade is implicated in the action of growth factors but also in the action of hormones such as endothelin and TSH.

REGULATION OF THE PLASMINOGEN ACTIVATOR SYSTEM IN CULTURED RAT ASTROCYTES

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Axotomy of the rat facial nerve leads to an increased expression of the tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) in astrocytes surrounding the injured motoneurons in the facial nucleus. We now report the regulation of tPA and PAI-1 synthesis in astroglial cells *in vitro* by various factors that play a potential role in mediating neuron-glia signalling in the central nervous system following injury: calcitonin gene-related peptide (CGRP), basic fibroblast growth factor (bFGF) and transforming growth factor-β1 (TGF-β1). The effects of these factors were compared with those of direct stimulation of second messenger pathways with forskolin and phorbol 12-myristate 13-acetate (PMA). Astrocyte-enriched cultures of neonatal rat brain were grown to confluency for up to 10 days. Changes in tPA-mRNA and PAI-1-mRNA levels were analysed by northern blotting and autoradiography following treatment with various substances over a time course of 2 to 32 hours. Stimulation with CGRP or bFGF resulted in an increase of both tPA-mRNA and PAI-1-mRNA production with a maximum at around 4 h, similar in time course to the effect of PMA. However, the effects of phorbol ester treatment were up to 10-fold stronger than those of CGRP and bFGF. TGF-β1 had differential effects on tPA and PAI-1: while PAI-1-mRNA synthesis was stimulated as strongly as with PMA, tPA-mRNA was decreased to about half the basal level. Forskolin completely abolished PAI-1-mRNA production. These findings point to a potential role for these factors in regulating the expression of tPA and PAI-1 in astrocytes following motoneuron injury.

DEVELOPMENT OF GLIA IN THE EMBRYO OF *DROSOPHILA MELANOGASTER*

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At the end of embryogenesis the CNS of *Drosophila* consists of a highly ordered array of neurons and glia. What are the mechanisms leading to a glial versus neuronal cell fate ? As a first step towards approaching this question we have made an almost complete description of the glial population in the ventral nerve cord of the first instar larva using glia specific enhancer trap lines. Using these lines it was possible to trace most of the identified glial cells back to early stages and at least in some cases to their precursors. It turned out that most of the cells show a specific migration behaviour which leads to their final distribution. Furthermore, cell divisions could be observed at certain developmental stages presenting information about clonal relationships of identified glial cells. Three of these lines (692, 2138, 3702, a generous gift from A. Spradling) labelled the whole population of identified glia except the midline glia which derives from special midline precursors. All three enhancer trap lines are homozygous lethal P-element insertions in 90 F1-4 affecting a gliaspecific gene which codes for a homeobox protein (D. Halter, personal communication). Our preliminary analysis of the mutant phenotype of the most severe allele suggests a nearly 50 % reduction of glial cell number at late embryonic stages and irregular distribution of these cells. Therefore it is likely that this gene plays an important role in the differentiation of glia in the *Drosophila* embryo.

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INDUCTION OF C- FOS IMMUNOREACTIVITY IN GLIAL CELLS FOLLOWING A CHEMICALLY INDUCED DEMYELINATION OF THE ADULT MOUSE SPINAL CORD.

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The immediate early expression of the C-Fos gene can be triggered in many cell types by numerous stimuli and represents a non specific cellular activation which can lead to a variety of cellular changes depending upon the cell types (from proliferation to differentiation). A model of acute demyelination by injection of lysophosphatidylcholine (LPC) in the adult mouse spinal cord has been used to test the ability of myelin forming cells to migrate and remyelinate when grafted at distance from this lesion (Gout et al., Neurosci. Lett., 89, 195-199, 1988). These grafted cells were able to migrate up to the lesion on defined pathways (Baron- Van Evercooren et al., J. Neurosci. Res., 35, 428-438, 1993; Vignais et al., Int. J. Dev. Neurosci., 11, 5, 603-612, 1993). During spontaneous remyelination of the LPC lesion data suggested that host's glial cells were recruited by the lesion (Tourbah et al., J. Neurosci. Res. 33, 47-59, 1992) and reexpressed transiently the embryonic form of the NCAM (Nait Oumesmar et al., J. Neurochem. Vol. 61 supplement, S 109 C, 1993) which could favour migration and remodeling in the lesioned site. In this study, we analyzed by immunohistochemistry (IHC) the expression of the immediate early C-Fos protein at various times (30 minutes to several days) after LPC induced demyelination of the adult mouse spinal cord. Responsive Fos-positive cells were found up to 2 mm in the ipsilateral side of the lesion, equally in grey and white matter and farther in the rostral than in the caudal direction.The intensity of C- Fos labelling of these cells decreased with their removal from the lesion.By using double IHC, astrocytes and oligodendrocytes appeared differently affected by the lesion. Indeed, the Fos responsive astrocytic population largely overlapped the area of Fos-positive oligodendrocytes which surrounded more closely the center of the lesion, suggesting that the mecanisms by which these two cell types are activated may be distinct. Moreover, in the lesioned area, not all cells of a given cell type were Fos immunoreactive. Other cell types, such as neurons and cells along the ependymal wall also expressed the c-Fos related proteins after injection. This expression of the c-Fos transcriptional factor by glial cells consecutively to the LPC induced demyelination may be a prerequisite for later events (reexpression of PSA NCAM, gliosis, proliferation, remyelination...) involved in glial plasticity and remodeling of the injured area.

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DETECTION OF CIRCULATING TUMOR NECROSIS FACTOR (TNF-α) AFTER EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE) INDUCTION IN LEWIS RATS

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Cytokines may play key roles in the regulation of myelin autoimmune responses, as well as in mediation of inflammatory myelin destruction. Studies of cytokines are hampered by their mutual interdependence and their rapid catabolism in body fluids. Another complicating event in the study of cytokines is the presence of soluble forms of specific receptors which role in immunological reactions is not clear. We studied the level of TNF cytotoxic activity in the sera and cerebrospinal fluids from Lewis rats after EAE induction with purified guinea pig myelin. We have also investigated the localization of TNF expression in the CNS by immunohistochemical methods. Soluble TNF activity was estimated by L929 fibroblast bioassay. Results were expressed in U/ml in reference to the cytotoxic activity of a standardized (Genzyme) preparation of recombinant human TNF-α. 1 unit representing about 50 pg human TNF. This cytotoxic method detects concentrations of TNF above 0.5 U/ml. The data obtained showed that EAE induction results in detectable circulating levels of biologically active TNF. TNF production was well correlated with encephalitogenic effect. Our observations demonstrated that circulating TNF is detectable from the third day after EAE induction , before the onset of clinical responses. The higher concentrations of TNF bioactivity were obtained days 11-12, in relation to the clinical symptoms. Immunohistochemical studies showed release of TNF-α by monocytes and macrophages. Other CNS cellular components could be involved in the expression of TNF and are currently being investigated.

ENDOPEPTIDASE 3.4.24.16 IN NEURONS AND ASTROCYTES

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The mechanisms by which neurotensin was inactivated in homogenates of primary cultured neurons and astrocytes indicated that endopeptidase 3.4.24.16 (E:24.16) contributed to neurotensin proteolysis in both cell types. These two cell systems were therefore used to examine various physicochemical and physiological properties of the enzyme. Astrocytic and neuronal E:24.16 exhibited distinct features that were the following : 1) all astrocytes in culture appeared immunopositive for E:24.16 while the proportion of neurons that were recognized by the anti-E:24.16 IgG fraction decreased along with the differentiation process. This was accompanied by an important concomitant increase in the neuronal E:24.16 activity, suggesting an overexpression of the enzyme during the maturation of neurons. By contrast, the activity did not vary between 2 weeks and 2 months in astrocytes. 2) Plated astrocytes could not hydrolyse a quenched fluorimetric substrate (QFS) of E:24.16 while neurons could efficiently cleave this substrate. This indicated that a membrane-bound form of E:24.16 with the catalytic site located on the external side of the plasma membrane occured in neurons but not in astrocytes. 3) At the electron microscopic level, we established that neurons displayed an intracellular but also a membrane-bound form of E:24.16 while the latter form totally lacked in astrocytes. 4) Interestingly, the membrane-bound versus cytosolic ratio of the activity underwent a ten-fold increase from day 0 to day 8 of cultures in neurons. This seemed to suggest a targetting of E:24.16 to a membrane-bound compartment during differentiation of primary cultured neurons. 5) Finally, astrocytes could constitutively secrete E:24.16 while neurons did not.

INDUCTION OF NITRIC OXIDE SYNTHASE AND INTERLEUKIN-1ß IN MICROGLIAL CELLS: POSSIBLE INTERACTIONS BETWEEN MICROGLIA AND ASTROGLIA.

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During development of the brain, activation of glial cells has been demonstrated to be essential for normal neuronal proliferation and differentiation. Simultaneous neuronal cell death has been demonstrated during this period.
Astroglial cell proliferation and differentiation is dependent on production of cytokines by microglial cells i.e. IL-1. In response to the activation by cytokines the astroglial in turn secrete factors which have proliferating effects on microglial cells and support neuronal survival. In contrast glial cells can produce nitric oxide which may be important during brain development causing neuronal cell death. Using rat glialmix cultures and purified cultures of astroglia and microglia, the production of nitric oxide synthase and interleukin-1 after endotoxin challenge has been studied in vitro.
The microglial and astroglial cells have been isolated after a two week co-culturing period after which microglia cells can be purified using a shaking procedure based on differences in adherence capacity of the two types of glial cells. The purity of the microglia after this procedure is 97% and after selectively killing of the remaining adhaerent microglia cell population, an astrocyte population can be obtained with a purity of 98%. The cells have been characterised using isolectin staining for microglial cells and GFAP staining for astroglial cells. The cultures are incubated with 100 µg/ml LPS (E. coli, Difco batchno. 055B5) for 24 hours followed by fixation with 4% paraformaldehyde for 30 min. The inducible nitric oxide synthase (iNOS) and interleukin-1ß (IL-1ß) have been demonstrated in our cultures by immunocytochemical staining using antibodies against inducible nitric oxide synthase and interleukin-1ß. In glialmixed cultures of microglia and astroglia we have demonstrated that (1) only the microglial cells and not astroglial cells are responsible for the production of iNOS and IL-1ß after endotoxin challenge. (2) A time-dependent decrease of the number of iNOS positive microglial cells has been observed in the glialmix culture at day 6 and 8 when compared to the number of IL-1ß positive microglial cells in glialmixed cultures containing increasing numbers of microglia and astroglia. (3) However when the microglial cells are isolated 8 days after coculture, all cells are immunopositive for iNOS and IL-1ß. In conclusion in the presence of increasing amounts of astroglial cells, the microglial cells appear to be inhibited in the production of iNOS but not of IL-1ß. This apparent interaction between astroglial and micoglial cells on the production of nitric oxide is currently under investigation.

*Frank's unexpected death leaves us with the memory of an inspiring teacher

EARLY AND LONG TERM EFFECTS OF INTERFERON-γ (IFN-γ) ON INWARD-RECTIFYING K^+ CHANNELS IN RAT MICROGLIA

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IFN-γ is known to induce or prime microglial cells to enter into the so called activated state. From an electrophysiological point of view its only known effect on microglial cells is the enhanced expression of outward-rectifying (OR) K^+ channels, induced by a long term application.

In the present investigation we studied the effect of acute and chronic treatment with IFN-γ on inward- and outward-rectifying K^+ channels expressed by cultured, purified rat microglial cells. We found that long term treatment (24 hours) with 10 u/ml IFN-γ caused not only an enhanced expression of OR K^+ channels, but also an increase of the inward-rectifying (IR) K^+ channel density. On the other hand, acute application of IFN-γ (10-100 u/ml) produced a negative modulation of the IR current. Such an effect was detectable after 1 min application and reached a maximum (79±17 % inhibition) at 5 min. In order to assess a possible involvement of protein kinase-C (PK-c) on the above mentioned responses to IFN-γ, we found that phorbol-myristate-acetate, a PK-c activator, was able to mimic the acute and chronic IFN-γ effects on the IR currents. Taken together these observations reveal two not yet documented effects of IFN-γ treatment: **a)** a long term enhancement of IR channel density; **b)** an early inhibitory modulation of IR currents. Interestingly both these effects seem to occur though a mechanism involving PK-c.

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EVIDENCE FOR THE INVOLVEMENT OF H^+-ATPASES IN I.C. pH-REGULATION OF GLIAL CELLS

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Several mechanisms are involved in cytosolic pH regulation of glial cells. The roles of the Na^+/H^+ exchanger, Cl^-/HCO_3^- exchanger and Na^+/HCO_3^- cotransporter has been studied before in detail. Less is known about the contribution of H^+-ATPases. The existance of such proton pumps has been demonstrated for several cell types, such as epithelial cells, or macrophages. In this study, the role of H^+-ATPases in glial pH homeostasis was investigated, using the pH-sensitive fluorescent dye BCECF as an indicator of intracellular pH. H^+-ATPases can be inhibited by N-ethylmaleimide (NEM). Application of 1 mM NEM leads to a cytosolic pH decrease of 0.4 - 0.5 units within ten minutes in C6 glioma cells, and of 0.2 - 0.3 in rat astrocytes. Within 60 minutes a partial recovery was seen. Further studies were conducted to identify the H^+-ATPase subtype involved. Three subtypes are known: The F_0F_1(mitochondrial) H^+-ATPase, E_1E_2-type ATPases, and vacuolar type H^+-ATPases. As NEM blocks all of these subtypes, a more specific inhibitor was required for further investigation. Application of 50 µM 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD-Cl), a specific inhibitor of the vacuolar type H^+-ATPase, also caused an acidification, which was similar to the effect of NEM. Thus, it can be concluded, that the membrane of glial cells possesses H^+-ATPases of the vacuolar type which might play a role in the regulation of intracellular pH. Another explanation would be a contribution in acid extrusion mechanisms at the blood brain barrier.

INTRACELLULAR ACIDIFICATION OF GLIAL CELLS BY QUERCETIN, AN INHIBITOR OF LACTATE EXPORT

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C6 glioma cells as well as astrocytes from primary culture exhibit a well reproducible, and statistically significant intracellular (i.c.) acidification (0.41 pH units) without recovery when exposed to the flavonoid quercetin (50 µM), as determined from the ratio of BCECF-fluorescences at 442 and 492nm. The maximum of the pH-decrease is reached after 16 min. The kinetics of acidification compare well with the swelling kinetics observed in an earlier study (1) after quercetin (25 µM): 13% glial swelling were found with a plateau reached after 15 min. Iodoacetate, an inhibitor of glycolysis, prevented swelling. Swelling and i.c. acidification are accompanied by a significant ($p<0.025$) 50% reduction of lactate release into the extracellular medium. Glial cells produce considerable amounts of lactate in-vitro, and probably also in-vivo. The observations are in line with the contention (1) that quercetin - among other effects - inhibits a glial lactate export system. As a consequence lactate and H^+ accumulate within the glial cell, thereby reducing i.c. pH, and, by osmotic forces, contributing to the volume increase. Currently it is investigated whether the lactate retained in the cell is further metabolized to amino acids.

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SPHINGOMYELIN SYNTHESIS IN CULTURED OLIGODENDROCYTES

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Previous studies with fluorescent ceramide analogs[1] prompted us to identify the pathway and subcellular site of synthesis of sphingomyelin in oligodendrocytes. Pulse-chase studies with [^{14}C-methyl]-choline and [1,2-^{14}C]-ethanolamine revealed that phosphatidylcholine (PC) is the major phospho-choline donor to ceramide, in a phosphatidylcholine-ceramide exchange reaction (Kennedy pathway) to yield sphingomyelin (SM). Furthermore, preliminary experiments indicate that after full differentiation of the oligodendrocytes, at a stage when the bulk of membranous extensions are already formed, an alternative pool of PC, not synthesized via the CDP-choline pathway but rather via N-methylation of phosphatidyl-ethanolamine (PE) becomes an important source of phospho-choline for SM. Whether the pool of PE destined to become methylated to PC is exclusively derived via decarboxylation of PS is currently under investigation. Another striking observation was that after choline-labeling, the SM-pool with long chain fatty acids was labeled predominantly. Experiments to localize SM-synthesis in the purified oligodendrocyte plasma-membrane/myelin fractions are in progress. These results demonstrate that the oligodendrocyte is capable of switching to another pool of PC for SM synthesis in relation to its differentiation. When bulk amounts of membrane is synthesized, PC formed via the CDP-choline pathway is utilized for SM synthesis, and at a stage when the bulk of membrane is already formed, the renewal of SM utilizes a pool of PC which is formed by methylation of PE. This phenomenon might relate to restoration of the phospholipid composition of the plasma- and/or myelin membrane after signal transduction events at the plasma membrane.

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FLUORESCENT CERAMIDE ANALOGS ARE USED BY CULTURED OLIGODENDROCYTES. EVIDENCE FOR MULTIPLE SITES FOR SPHINGOMYELIN SYNTHESIS.

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We studied the metabolism of sphingolipids by oligodendrocytes derived from rat spinal cord by providing lipid vesicles with either N-lissamine-rhodaminyl-ceramide (LRh-Cer) or N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-ceramide (NBD-Cer) to the cells cultured in a chemically-defined medium. With both probes the major fluorescent product turned out to be sphingomyelin (SM). Most of LRh-SM was not cell-associated but recovered from the culture medium. The accumulation of LRh-SM, both in the cells and in the medium, was inhibited in the presence of monensin or brefeldin A, whereas the production of NBD-SM was much less affected by these Golgi perturbing drugs. Monensin treatment resulted in a shift from sphingophospho- towards sphingoglycolipid (GlcCer) synthesis. With LRh-Cer as substrate, LRh-labeled fatty acid (FA), galactosyl- and sulfogalactosyl-ceramides (GalCer and SGalCer) were also formed. NBD-Cer, however, was metabolized to glucosylceramide (GlcCer) and GalCer but not to SGalCer or NBD-FA. These data demonstrate that chemical modifications of ceramide alter its metabolism in oligodendrocytes and that the metabolites of LRh-Cer reflect the glycolipid composition of myelin more closely than those of NBD-Cer. Recent experiments with radio-labeled ceramides showed that a [^{3}H]-α-OH-ceramide is efficiently utilized for the synthesis of GalCer and SGalCer, in contrast to non-OH-ceramides. Our results with the fluorescent ceramide analogs indicate that SM-synthase does not completely co-localize with the enzymes that catalyse the synthesis of GlcCer, GalCer and SGalCer. Moreover, LRh-SM was found in the medium even during low temperature incubation with LRh-Cer (conditions known to reduce/block vesicular transport). We conclude that besides the *cis-medial* Golgi localization of SM synthase, there is a second site of synthesis which is distal to the *medial*-Golgi, likely to be the plasma membrane (or a related endosomal compartment).

TROPISM OF SEROTONERGIC NEURONS TOWARDS GLIAL TARGETS IN THE RAT EPENDYMA .B. VOUTSINOS , M. DIDIER-BAZES and M.F. BELIN. INSERM CJF 90-10, Lab. Anat. Pathol., Fac. Alexis Carrel, 69372 Lyon Cedex 08, France.

During development, recognition mechanisms between neurons and their targets are necessary for the formation of the neuronal network. Neural connections are synaptic or non-junctional. Both types of communication can be found between neurons and glial elements in the periventricular walls. Serotonergic (5HT) fibers form synaptic contacts on the specialized ependymocytes of the subcommissural organ. (SCO), a structure which form the roof of the third ventricle at its junction with the aqueduct. A network of non-junctional fibers containing both GABA and 5HT spread between the cilia of the classical ependymocytes (Ep.) in the ventricles. These anatomical, morphological and biochemical features suggest a tropism and specific recognition mechanisms between glial elements and 5HT neurons. This hypothesis can be tested by the study of the innervation of the SCO and the Ep. by grafted embryonic neurons after a chemical destruction of the 5HT endogeneous innervation. Solid implants or cell suspensions prepared from embryonic metencephalon were transplanted to either the third ventricle or the periventricular gray matter in 5,7 dihydroxytryptamine denervated rats. Grafted 5HT neurons were able to reinnervate the Ep. and the SCO. The fibers forming the supraependymal plexus were non-junctional and contained both 5HT and GABA while those innervating the SCO formed synaptic contacts and contained only 5HT. The signals capable of inducing the ependymal innervation were specific for 5HT neurons since catecholaminergic neurons present in the grafts were unable to innervate either classical or specialized ependymocytes. These results demonstrate that glial cells are targets for 5HT neurons and that the morphological and biochemical characteristics of the 5HT innervation are closely related to the target cell phenotype.

Analysis of the autoimmune response to Myelin associated glycoprotein (MAG) in the Lewis rat.

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Although autoimmune T cell responses to MAG have been reported in patients with multiple sclerosis, it is not known whether they play a role in the pathogenesis of this disease. We therefore investigated the pathogenicity of MAG-specific T cell response in an animal model, EAE. Initial studies used synthetic rat MAG peptides spanning predicted T cell epitopes. The adoptive transfer of rat T cell lines recognising two of these peptides, [a.a. 119-137 (extracellular) and a.a. 561-582 (carboxyl terminus of S-MAG)] induce a subclinical encephalomyelitis in Lewis rats, demonstrating for the first time that MAG-specific T cells can indeed induce an inflammatory response in the CNS. However, further analysis of this autoaggressive immune response required large amounts of the entire polypeptide. We therefore generated a panel of prokaryotic MAG fusion proteins corresponding to both the complete sequences of L-MAG and S-MAG, as well as proteins of the extracellular domain (E-MAG) and adjacent pairs of the IgG-like domains 2 and 3 (R2), 3 and 4 (R3), and 4 and 5 (R4). The resulting fusion proteins, expressed in the vector pQE 12 (Quiagen), contain the appropriate MAG sequence together with an additional N-terminal tripeptide (RGS) and a C-terminal poly-histidine tail (GSRHHHHH). Preliminary experiments using analogous fusion proteins indicate that the "foreign" residues do not influence the immunogenicity/ pathogenicity of the fusion proteins in rabbits or Lewis rats. The expressed MAG fusion proteins will prove further the pathogenicity of the MAG-specific immune response *in vivo*, as well as providing further tools to investigate the function of this protein.

FUNCTION OF THE POU-DOMAIN PROTEIN TST-1 DURING GLIA-SPECIFIC GENE EXPRESSION

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Tst-1 which is also known as Oct-6 and SCIP is expressed during the development of myelinating glia in the peripheral and the central nervous system. To better understand its function as a transcriptional regulator in these cells we analyzed its impact on the expression of genes specifically expressed in myelinating glia. We found that Tst-1 functions not only as a transcriptional repressor as exemplified by its influence on the Po gene, but also as a transcriptional activator of glia-specific genes.

A convenient system for analyzing the stimulatory role of Tst-1 is the human papovavirus JC, the etiologic agent of PML. This fatal demyelinating disease of the human brain is caused by the cytolytic destruction of oligodendrocytes which are specifically targeted by the virus. Tst-1 activates expression from both viral promoters 5-10 fold by binding to defined A/T-rich elements within the viral regulatory region. One of the viral proteins induced by Tst-1 is JC T-antigen, which not only functions as a central regulatory molecule during viral DNA replication, but is itself a potent regulator of gene expression. Interestingly, once JC T-antigen is produced, it can synergistcally interact with Tst-1 to increase viral gene expression even further. Transcriptional cooperativity between both proteins is likely due to direct physical interaction involving the aminoterminal region of JC T-antigen and the POU-domain of Tst-1. We believe that the observed synergism between glial transcription factor and viral regulatory molecule might be an important aspect in the pathophysiology of PML.

ASTROCYTIC DAMAGE IN PROLONGED CORTICAL ISCHEMIA IS DEMONSTRATED BY ALTERED IMMUNOLABELLING FOR GFAP

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Astrocytes can be damaged together with neurons in prolonged ischemia (cerebral infarction). We have used immunohistochemical labelling with antibodies to glial fibrillary acidic protein (GFAP) to characterize ischemic damage of astroglia in two rat models. Photochemically-induced thrombosis induced focal cortical ischemia. Astrocytes showed a particular pattern of disintegration with a massively enhanced GFAP signal with polyclonal antibodies, beginning after 30-60 min of ischemia. Acute changes within the core of the developing infarcts were different from swelling-induced increases of cellular labelling seen in the surround during the first 24 h. Reactive astrocytes demarcated the infarct at 2-3 days. Mildly enhanced labelling for GFAP was also seen in the entire affected hemisphere for at least 3 days. Global ischemia was produced by immediate post-mortem anaerobic incubation of brain tissue at 37 °C. At 2 to 4 h, a massive increase of GFAP labelling was seen in astrocytes located in the outer cortical layers. In both conditions, a monoclonal antibody changed in labelling from moderate control signals to negative. In summary, we have now established criteria for GFAP-immunohistochemistry as a marker of acute astrocytic damage in prolonged cerebral ischemia.

BUTYRYLCHOLINESTERASE AS A MOST POTENT REGULATOR OF AVIAN SCHWANN CELLS IN VITRO.

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Butyrylcholinesterase (BChE) is expressed before outgrowth of motoraxons (Layer 1990), during regeneration of crushed peripheral nerves, and during pathological states including Alzheimer's disease or gliomas. Since a glial origin of BChE in all these cases seems likely, we here compared the relation of chicken Schwann cell (SC) proliferation with their capacity to express both types of cholinesterases in vitro.

Schwann cells were prepared from chicken sciatic nerve to establish short-term and long-term cultures. Instead of using antimitotics like Ara C to suppress fibroblast proliferation, we have implemented a new selective medium for Schwann cells to result in pure SC cultures. These cultures were stained histochemically for BChE (Tago et al. 1986). Staining was localized to central cytoplasm and nuclei of Schwann cells, presumably during their mitosis. Cocultures of SC and DRG cells showed that SC retain normal functionality like myelination.
Long-term cultures were used to study the influence of several factors including cytokines, growth factors and BChE on the rate of proliferation and expression of cholinesterases. IFN-gamma reduced proliferation of SC while simultaneously increasing expression of both AChE and BChE. Similarly, Interleukin-1ß reduced cell proliferation, but slightly increased BChE while reducing the amount of AChE. Forskolin had only little effects on proliferation and AChE, but noticeably was able to double the amount of BChE. Pituitary extract containing the glial growth factor GGF again was not mitogenic, but increased both cholinesterases by about twofold. Laminin, another mitogenic factor for SC, failed in our experiments, but stimulated BChE significantly. Most noticeably, the most potent agent to stimulate both cell proliferation and expression of BChE (up to 4fold) was BChE itself. These strong effects are seen both in serum-containing and in a complex medium, and can be counteracted by the BChE inhibitor iso-OMPA.
These data suggest a prominent role of BChE in SC regulation. They further support our hypothesis on regulatory functions of BChE in cell proliferation, neurite outgrowth, differentation and regeneration (Layer et al. 1993, Willbold and Layer 1992).

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The soluble form of intercellular adhesion molecule-1 (sICAM-1) is present in the supernatants of activated cultured human umbilical vein endothelial cells (HUVEC) and astrocytes as well as in cerebrospinal fluid (CSF) of patients with bacterial meningitis

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Previously, an increase of ICAM-1 expression was demonstrated on the surface of HUVEC after stimulation with various cytokines, LPS and CSF from patients with bacterial meningitis. This increase of ICAM-1 expression was also detected for cytokine stimulated astrocytes.
In this context it was a case of interest, whether the production of sICAM-1 can be induced by stimulation as described below.
The concentration of sICAM-1 in supernatants of HUVEC and astrocytes as well as CSF of patients with bacterial meningitis was measured by enzyme immunoassay (EIA).
Stimulated by cytokines or LPS, the sICAM-1 concentration in the supernatants of HUVEC rose continuously depending on the concentration of the stimulus and time of incubation, until a plateau was reached. TNF-alpha showed the greatest stimulatory effect and after 24 hr exposure to TNF-alpha the maximum concentration of s-ICAM-1 produced by HUVEC was nine times higher than that without stimulation. The expression of sICAM-1 increased five-fold during stimulation with IL-1ß and six-fold when stimulatd by LPS.
In the supernatants of cytokine stimulated astrocytes only a small concentration of sICAM-1 was detected. The supernatants of HUVEC which were stimulated with CSF of patients with bacterial meningitis showed increased sICAM-1 concentration and after 24 hr exposure the sICAM-1 concentration in the CSF/supernatant-solution was doubled. Only small sICAM-1 concentrations were measured in CSF of patients without inflammatory diseases of the CNS (mean: 1,04 ng/ml; range: 0,01-2,06 ng/ml) whereas in CSF of patients with bacterial meningitis the values where significantly increased but did not depend on the species of the bacteria (mean: 40,24 ng/ml; range: 3,55-225,03 ng/ml).

STRUCTURAL CHARACTERIZATION OF MURINE MYTI, A ZINC FINGER GENE EXPRESSED IN THE OLIGODENDROCYTE LINEAGE

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MyTI, a novel gene of the zinc finger superfamily of transcription factors, binds to the cis-regulatory region of the myelin proteolipid protein (PLP). MyTI is most highly expressed in the developing nervous system, where expression precedes induction of its presumptive target, PLP. A novel structural zinc finger element has been found in MyTI: three cysteine and one histidine residue (C2-HC) are coordinated in a zinc dependent manner. The six zinc finger domains are highly conserved and occur in two widely separated clusters of two and four fingers each [1]. MyTI is encoded by a single gene, but a zinc finger family member that is closely related to MyTI was also found to be expressed in early CNS development. This MyTI family member displays 92% amino acid identity with MyTI in the DNA binding domains.

In order to investigate the functional role of MyTI in myelination and development, we are constructing transgenic mice with a knock-out mutation for MyTI. We have screened a 129SV genomic phage library with a human cDNA probe for MyTI and have characterized a genomic region containing the proximal two-finger domain of MyTI. This domain is encoded by three exons, with an intron disrupting the second finger unit. The coding regions of the mouse MyTI are highly homologous to the human MyTI (90% amino acid identity, 84% nucleotide identity). A targeted mutation will be created in transgenic mice by use of a construct carrying the genomic MyTI with the insertion of the lacZ gene and the neo and tk selectable markers. By means of homologous recombination MyTI will be rendered dysfunctional and its expression through development will be monitored by β-galactosidase activity. The effect of MyTI on myelination and its role as a myelin-specific transcription factor can then be evaluated.

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CYTOKINES, PROSTAGLANDINS AND LIPOCORTIN-1 ARE PRESENT IN THE BRAINS OF SCRAPIE-INFECTED MICE

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The neuropathology of scrapie and Creutzfeldt-Jakob disease is characterised by chronic neurodegeneration with vacuolation of neurons and/or neuropil and amyloid plaque formation in some cases. A classical acute inflammatory response and immunopathology in the form of perivascular cuffs are absent. A modified, protease-resistant isoform (termed PrP^{sc}) of the host-encoded PrP protein accumulates in those areas of the brain showing vacuolation and plaques; astrocyte hypertrophy and proliferation and a marked microglial activation are also present in these areas. The microglia show upregulation of leukocyte antigens with known functions in recruitment, phagocytosis (CR3), leukocyte activation (LCA) and antigen presentation (MHC II) which suggests that the microglial response represents a modified inflammatory response. In the present studies, the presence of cytokines, prostaglandins and lipocortin-1 was investigated in terminally-affected mice of two scrapie models, using immunocytochemical techniques. There was marked induction of interleukin-1ß, tumour necrosis factor α, prostaglandin (PG) E_2, $PGF_{2\alpha}$ and lipocortin. Although the distribution of immunoreactivity differed between the two models, the pattern of staining within each model was similar for all factors examined and corresponded to areas of the brain showing vacuolation of neurons/neuropil, astrocytosis and microglial activation. A comparison of the morphology of cells expressing these factors with GFAP (astrocytic) and F4/80 (microglial) staining showed that they occurred predominantly in astrocytes. It has previously been proposed that cytokines influence or mediate abnormal APP processing and ßA4 deposition. The findings reported here indicate that similar mechanisms may also be relevant in PrP processing and the pathogenesis of neurodegeneration in scrapie.

MORPHOLOGICAL CHANGES IN THE SUPERIOR COLLICULUS OF THE RAT AFTER DEAFFERENTATION

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In contrast to the peripheral nervous system regeneration after lesions does not occur in the central nervous system (CNS) of adult mammals. However, if growth inhibitory aspects of the CNS environment are neutralized, axons of retinal ganglion cells (RGC) can regrow *in vitro* and *in vivo*. After reaching their target neurons in the adult superior colliculus (SC) regenerating axons may even form functional synapses with their target cells.

During embryonic development of the vertebrate visual system an ordered projection from the retina onto the SC is established. We have recently shown that optic nerve transection induces putative guidance activities for temporal and nasal retinal axons in the SC of adult rats with a delay of 7 - 14 days after lesion.

In the present study we investigated morphological changes in the cellular compartment of the SC after optic nerve lesion. Different antibodies which identify glial cells (GFAP, Vimentin and MBP), neurons (SMI-31) and microglial cells (OX-42) were used to determine whether distinct cell populations show delayed reactive changes.

1-2 days after optic nerve axotomy the GFAP expression in mature astrocytes was increased in the whole SC without regional preference. Simultaneously OX-42 positive microglial cells appeared in the afferent optic tract and anterior part of the SC. Furthermore the expression of MBP and SMI-31 decreased in a rostro-caudal direction. Astrocytes started to express Vimentin 1 - 2 weeks after lesion.

In conclusion, all cell populations examined in the SC showed 'reactive' changes after optic nerve transection. Thus, different factors might be involved in the reexpression of specific guidance information. However, the dedifferentation of astrocytes as indicated by their delayed expression of Vimentin points to this cell class as a possible candidate for the production of guidance molecules.

TUMOR NECROSIS FACTOR α (TNF-α) AS A MEDIATOR OF HUMAN OLIGODENDROCYTE DEATH BY ACTIVATED MICROGLIA.

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TNF-α has been implicated in the neuropathogenesis of several demyelinating diseases, including AIDS dementia complex and multiple sclerosis. Elevated TNF-a message levels in the brain parenchyma correlates with the severity of AIDS dementia (Wesselingh et al., *Ann. Neurol.*, **33**, 576, 1993) while TNF α protein is detected in active demyelinating lesions of multiple slerosis patients (Hofman et al, J. Exp Med, 170, 607 (1989).

Purified populations of human oligodendrocytes (>95% galactocerebroside positive) and microglia (>over 95 % positive for low-density lipoprotein receptor) were obtained from fresh temporal brain tissue of patients undergoing surgery for intractable epilepsy. When cultured, human microglia became activated and synthezised mRNAs for several cytokines including TNF-α. TNF-α protein synthesis by microglia was detected by immunoprecipitation of the 17 kD monomeric and 51 kD trimeric forms in cell extracts and by elisa assay on the cells supernatant. Cultured human oligodendrocytes (HO) regenerated their processes in response to PDGFαβ, IGF-1 and bFGF. RT-PCR analysis of oligodendrocyte total RNA demonstrated the presence of the TNF-α receptor-1 death domain sequences.

To investigate the cytotoxicity of human microglia for oligodendrocytes, we first used the CG4 rat oligodendrocyte (RO) cell line as target and stained with MTT, a dye which detects mitochondrial activity. Conditioned medium (CM) from human microglia caused up to 30% RO death, as observed with maximal doses of rhTNF-α, and this effect was neutralized by TNF-α antibodies. Moreover addition of live microglia at a ratio of 5 microglia to one RO caused similar cytotoxicity largely neutralizable by TNF α antibodies. When purified HO were used as the targets, dose-dependent cytotoxicity was observed in response to rhTNF-α while microglia-derived TNF-α caused ~ 25 % HO death. Activated human microglia co-cultured with HO was most potent in causing dramatic retraction of processes followed by death in up to 40 % of the cells. Again such effects could be largely neutralized by TNF-α inhibitors such as TNF-α antibodies and pentoxifylline.

These studies extend previous observations in the rat and clearly demonstrate that TNF-α derived from activated microglia can cause oligodendrocyte damage and death. The ability to attenuate this effect *in vitro* with TNF-α inhibitors provides a basis for therapeutic intervention in demyelinating diseases which may be TNF-α mediated. More over our *in vitro* models allows to further investigate the molecular basis of TNF-α neuropathogenesis.

THE LAMINA CRIBROSA OF THE RAT VISUAL SYSTEM. MORPHOLOGICAL AND IMMUNOCYTOCHEMICAL PROPERTIES

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The lamina cribrosa (LC) is an unmyelinated part of the optic nerve and believed to arrest the migration of oligodendroblasts from the optic nerve into the retina. The astrocytes have been suggested to play a role in this function, because they differ from astrocytes in other parts of the CNS in a number of properties: they are oriented mainly perpendicular to the longitudinal extension of the axons, their surface is extremely folded, the perivascular space is enlarged, the blood-brain barrier is leaky. We have found that the polarity of LC astrocytes in terms of the distribution of orthogonal arrays of particles is reduced as has been shown previously in the myelin-deficient rat, too. In addition, in juvenile rats we found neuritic varicosities which were in close contact to rows of astrocytes and absent in the optic nerve proper. Later in development, the astrocytes contained inclusion bodies which are tentatively suggested to be the product of phagocytosis of the neuritic varicosities by the LC astrocytes. These inclusion bodies were lacking in the optic nerve proper. Furthermore, in order to study closer the properties of the blood-brain barrier in the LC, we tested the occurrence and distribution of the glucose transporter GluT1 using a polyclonal antibody provided by Dr. W. Pardridge. We found that in contrast to the choroid and pial vessels around the LC the capillaries in the LC expressed GluT1 and revealed its asymmetrical distribution found also in the mature brain microvessels. Further work is needed 1. to demonstrate astrocytic phagocytosis of neuritic processes and 2. to investigate the permeability and other properties of LC microvessels. - With support by the DFG.

THE ASTROCYTIC NETWORK AS A MONITOR OF NEUROPIL IN THE CEREBRAL CORTEX OF ADULT RATS

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In adult visual cortex of rats, astrocytes and their processes, identified by immunohistochemistry for GFAP and by intracellular filling with HRP, were morphometrically analysed at light and electron microscopic levels. Data obtained confirm that about 17,500 astrocytes are distributed within 1 mm³ of tissue. According to this cell density an average tissue volume of 57,000 µm³ of each astrocyte corresponds to a tissue sphere with a diameter of 48 µm. However, direct measurements of astrocytic process spanning revealed a larger diameter (78 µm) of the tissue space per cell. The difference statistically quantifies the considerable degree of interdigitation (about 15 µm per radius) between processes of neighbouring astrocytes. The **interdigitation space** then represents 77 % of the tissue volume penetrated by an astrocyte and allows for a cooperative monitoring of the neuropil through neighbouring astrocytes. Surface and volume estimations showed that astrocytic lamellae (fine sheets of astrocytic processes) make up 90 % of the astrocytic surface membranes in this interdigitation territory. Hence high surface-to-volume ratios enable astrocytes to have a huge degree of exchange area with neighbouring cells and extracellular space. Thus 560 astrocytes may on average have contact to one cortical neuron. These morphometrical data suggest that the astrocytic network may intimately interact with the neuronal net.

CONTACT GUIDANCE OF O-2A PROGENITORS ON MICROFABRICATED SUBSTRATA

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In the developing CNS, oligodendrocyte-type 2 astrocyte [O-2A] progenitors migrate along naked axonal fascicles and eventually their processes ensheath and myelinate individual axons. The mechanisms underlying these events must be able to direct motility and outgrowth to produce an ordered pattern of axonal ensheathment. O-2A progenitors are surrounded by complex chemical, mechanical and topographical anisotropy, all of which may impart guidance information. We have investigated whether O-2A progenitors are contact-guided by surface topography by explanting cells derived from P7 rat optic nerve and P2 whole brain homogenates onto microfabricated substrata containing surface contours similar to those experienced by these cells in vivo. Microfabricated substrata were etched in quartz using a modification of the process used to make silicon wafer integrated circuits. These surfaces contain repeating patterns of square waveform and are devoid of chemical and mechanical cues. O-2A progenitors became highly aligned by surface contours of 1μm and repeat distances of 2μm periodicity. Their morphology was typically bipolar, elongated and constrained to ridge or groove contours. Processes of oligodendrocytes also demonstrated a striking parallel alignment in response to similar contours. As the repeat spacing between contours was increased to 4μm and above [depth of contours maintained constant] O-2A progenitors became less aligned and were able to bridge several repeat spacings. Astrocytes were also aligned by similar patterns of surface topography, some cells being entirely constrained by broad inter-groove spacings. Staining of intracellular F-actin using rhodamine-conjugated phalloidin revealed arrays of stress fibres in astrocytes but not in O-2A progenitors, suggesting that mechanical constraint of high order actin networks is not responsible for alignment of O-2A progenitors. Astrocyte stress fibres illustrated preferential alignment with the long axis of the surface contours, suggesting these structures may be involved in astrocyte contact-guidance. The distribution of F-actin in O-2A progenitors was diffuse and in oligodendrocytes appeared concentrated in patches within the arborised network of processes. We are currently investigating the lower limit of response to surface topography by using sub-micron contours, similar to those presented by individual axons.

THE ASTROGLIAL CELLS IN THE BASAL NUCLEI OF THE ADULT LIZARD.

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The present study describes the distribution of astrocytes in the basal nuclei of the adult lizard (Gallotia galloti) using both immunohistochemical methods and electron microscopy. GFA-P positive cell processes are numerous in the neuropil of the septal nuclei and ventral striatum but less abundant in the amigdala. In the striatum ventralis, the anterior commissure and amygdala there was intense glutamine synthetase (GS) immunoactivity in the cytoplasm and rough endoplasmic reticulum of astrocytes. Asrocytes in the basal nuclei contain scattered glycogen granules and gliofilaments, particularly in the processes. Satellite astrocytes were numerous. In conclusion, the astrocytes in the basal nuclei of the lizard may represent a specialised type of astrocyte. The astrocyte population of CNS of the lizard is as heterogeneous as that found in mammals (1) because in the midbrain of the same species two subclases of astrocyte were identified (2,3) and similar subtypes were found in the spinal cord of Lacerta lepida (4).

1. **Wilking et al., 1990. Tins 13, 43-46.**
2. **Monzón Mayor et al., 1990a. Glia, 12, 87-97.**
3. **Monzón Mayor et al., 1990b. J. Anat., 170, 33-41.**
4. **Bodega et al., 1990. Anat. Embryol., 181, 567-575.**

REACTIVE GLIOSIS: A CHARACTERIZATION.

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Local astrocytic response to brain damage represents a significant event in reorganizational process. Its modulation could optimize regenerative events. We describe application of an experimental procedure based on the semiautomated analysis of relative optical densities of GFAP + astrocytes after cerebral cortex and striatal damage in adult rats. Spongostan embedded in physiological saline or DMEM/F12 basal culture medium was inserted stereotaxically under pentobarbital anesthesia.Animals were sacrificed after 14/28 days and brains processed for GFAP immunocytochemistry. Images were fed into an image analyzer in 100 um epochs, starting at the edge of the lesion. "Basal" astrocytosis in intact animals was 2.35% in c.cortex and 3.87% in striatum. Astrocytic gradient in lesioned animals varied from 17.2% at 100um to 3.7% at 600um from the lesion. No significant regional differences were found, nor between the two control fluids administered.Coefficient of variation was 0.34. This procedure will be used to analyze the effect of locally administered substances.

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INTERACTION OF PEA-15, A NOVEL ASTROCYTIC PROTEIN KINASE C SUBSTRATE, WITH MICROTUBULES MAY BE REGULATED BY PHOSPHORYLATION.

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Previously, we have described a novel substrate of protein kinase C enriched in astrocytes, PEA-15 (J. Biol. Chem. 268:5911-5920) which is an intracellular target of norepinepherine. The protein exists in at least three forms, a nonphosphorylated form, N, a form phosphorylated on one site, Pa, and a form phosphorylated on two sites, Pb. To identify the function of the protein, antisera was raised against internal sequences of PEA-15. Indirect immunofluorescence revealed fibrous cytosolic staining in cultured astrocytes, suggesting that PEA-15 is a cytoskeletal protein. Consequently, astrocytes were double labeled using anti-PEA-15 antiserum and either phalloidin to reveal actin, antibodies against glial fibrillary acidic protein or ß-tubulin. The immunoreactivity of PEA-15 colocalized with that of ß-tubulin. To examine whether PEA-15 is associated with microtubules, astrocytes were treated with nocodazole to depolymerize tubulin. Nocodazole treatment resulted in diffused immunostaining patterns of both ß-tubulin and PEA-15. Subsequently, the drug was washed out to allow microtubules to reassemble. PEA-15 immunoreactivity also returned to the fibrous pattern and colocalized with microtubules. These data suggest that PEA-15 may interact with microtubules. To examine whether phosphorylation of PEA-15 regulates microtubules association, phosphorylation states of the protein in the unpolymerized tubulin fraction extracted using an nonionic detergent and in the polymerized tubulin fraction, the detergent extracted residue, were analyzed. N and Pa were found in the unpolymerized tubulin fraction whereas Pa and Pb were detected in the polymerized tubulin fraction. Furthermore, the depolymerization of microtubules by nocodazole increased the levels of N and Pa whereas the stabilization of microtubules by taxol resulted in an increase in Pb and a decrease in N. These observations suggest that phosphorylated forms of PEA-15 are associated with microtubules.

MONOCLONAL ANTIBODIES TO SULFATED GLYCOLIPIDS IN DEMYELINATING NEUROPATHIES : RELATIONSHIP BETWEEN TARGET ANTIGEN, CLINICAL PATTERN AND IMMUNOLOCALIZATION OF THE ANTIBODIES ON PERIPHERAL NERVE

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Antibodies to sulfated glycolipids have been mainly reported in patients with demyelinating polyneuropathies and IgM gammopathies. We report here our study on 40 cases of demyelinating peripheral neuropathy associated to IgM monoclonal antibodies. We showed that in 33 cases of them, the IgM reacted at high titer with the HNK-1 epitope carried by two sulfated glucuronyl-paraglobosides (SGPG/SGLPG). In 16 of those, the IgM reacted also at high titer with sulfatide. Immunohistochemistry study performed on frozen unfixed sections, provided from biopsies of human sural nerve, showed that IgM directed to both SGPG/SGLPG and sulfatide, were mainly localized on myelin, but IgM reacting only with SGPG/SGLPG bound essentially to Schwann cells. This was evidenced by indirect immunofluorescence and double staining method using a polyclonal antibody specific to Schwann cells (S-100) These results suggest a variability of the IgM anti-SGPG/SGLPG monoclonal antibodies and a difference in the fine structure of the epitope recognizing a different presentation of the antigen on the surface of cells. Our results suggest a correlation between the difference in the fine structure of the epitope recognized by the IgM and the difference in the pattern of immunolocalization of these IgM on peripheral nerve. Clinical data showed a correlation between the presence of anti-sulfatide antibodies and the intensity of pain.

NITRIC OXIDE SYNTHASE ACTIVATION IN NEONTAL RAT SCHWANN CELLS

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Nitric oxide (NO) is neurotoxic in vitro. We have detected the cytokine-inducible form of NO synthase (iNOS) by immunohistology in sciatic nerves of Lewis rats with adoptive-transfer experimental autoimmune neuritis (AT-EAN). While infiltrating macrophages contribute to iNOS expression in AT-EAN, a diffuse but specific background staining prompted us to search for iNOS in Schwann cells. Cultured neonatal rat sciatic nerve Schwann cells (95% pure, fibroblast-depleted, macrophages <1%) secreted nitrite, a reaction product of NO in aequous solutions, in a time-dependent fashion after in vitro stimulation with recombinant rat gamma-interferon (G-IFN, 100 U/ml) and, in higher amounts, after additional treatment with murine tumor necrosis factor alpha (TNF-alpha, 100 U/ml). IL-1 and A23187 had no effects. The cytokine-induced secretion of nitrite by Schwann cells was inhibited by N-monomethylarginine (NMMA), a competitive inhibitor of iNOS, but not by N-nitroarginine (NNA), an inhibitor of the constitutive neuronal and endothelial forms of NOS. In vitro, cytokine-activated rat Schwann cells suppressed antigen-induced proliferation of P2-specific T cells in a cell-dose dependent fashion. This suppression was reversed by adding NMMA. Our results suggest that NO may play a role in down-regulating autoimmune responses to antigens of the peripheral nervous system.

ULTRASTRUCTURAL LOCALIZATION OF THE E1 EPITOPE IN NEURON-GLIAL CELL INTERACTION

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E1 is a phylogenetically conserved glyco-epitope represented on a series of cell surface proteins of CNS and PNS glial cells as well as certain neuronal populations. During the development of dorsal root ganglia (drg) E1 antigen has been exclusively found on glial cells until embryonic day 7 (E7), while later on it appears also on a fraction of drg neurons. In single cell cultures from embryonic peripheral sensory ganglia E1-positive and E1-negative neurons display striking differences in their interaction with E1-positive glial cells. While E1-negative neurons grew associated and became ensheathed with E1-positive glial cells, E1 positive neurons avoid these contacts. Since NGF induced neurite outgrowth of E1 negative neuons could be completely blocked by adding a Mab against E1 to neuron-glia cultures, the E1 epitope might be relevant to neurite growth regulation. In the present study we analyzed neuron-glial contacts of E1 negative neurons with E1 positive glial cells immunocytochemically using double-immunofluorescence and confocal laser scanning microscopy or pre-embedding immunogold labelling at the ultrastructural level. Immunocytochemical detection by confocal laser scanning microscopy revealed numerous small patches of the Mab E1 which were scattered over many glial cell bodies and processes. At the electron microscopic level some of these E1 positive patches could be identified as neuron-glial contact zones where immunogold particles were present at sites of neuron-glia membrane contact.

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PMP22/GAS-3 MEDIATED GROWTH MODULATION IN SCHWANN CELLS AND FIBROBLASTS

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PMP22/gas-3, the proposed candidate gene for inherited peripheral neuropathies in mice (*trembler, tremblerJ*) and human (*CMT1a, HNPP, Dejerine-Sottas*) is thought to serve a dual function in cell growth and myelination. In order to elucidate the biological functions of PMP22/gas-3 we have used a combined molecular and cell biological approach to alter PMP22/gas-3 expression in vitro. By means of retrovirus-mediated gene transfer restriction fragments of a PMP22 cDNA have been transduced in sense or antisense orientation into secondary rat Schwann cells and the permanent fibroblast cell line NIH3T3. Gene transfer by infection results in the integration of PMP22 expression vectors into the host cell genome, as monitored by DNA-PCR analysis. The expression of transduced PMP22 cDNA sequences, investigated by Northern Blot, RNA-PCR and Western Blot analyses, mediates altered PMP22 expression in recombinant cells: sense PMP22 expression vector infected cells show enhanced PMP22 mRNA and protein levels, one antisense PMP22 expression vector significantly reduces total PMP22 mRNA levels. We have used these recombinant cells to investigate the putative role of PMP22 in cell growth. The effect of altered PMP22 expression levels, as monitored by BrdU incorporation based proliferation assays applied to non-synchronized cell cultures and flow cytometric cell cycle analysis of synchronized cell cultures, will be described and a function of PMP22 in cell cycle progression discussed.
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EFFECTS OF TRIMETHYLTIN ON GLIAL AND NEURONAL CELLS IN AGGREGATE CULTURES: DEPENDENCE ON THE DEVELOPMENTAL STAGE

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Trimethyltin (TMT), a well-known neurotoxicant, was used to study the influence of the developmental stage on the response of different brain cell types to a neurotoxic insult. Immature and differentiated aggregating brain cell cultures of fetal rat telencephalon were treated during 10 days with TMT at different concentrations, ranging from 10^{-10} M to 10^{-6} M. In immature cultures astrocytes showed increased staining for glial fibrillary acidic protein (GFAP), characteristic of gliosis, but no change in the activity of glutamine synthetase (GS). Neurons and oligodendrocytes appeared to be less sensitive, since a decrease in the activity of cell type-specific enzymes and in neurofilament protein content was observed only at 10^{-6} M of TMT. In differentiated cultures, changes in cell type-specific parameters occured only at 10^{-6} M of TMT. At this concentration, the effects of TMT on neurons were the same as for immature cultures, but responses of glial cells were found to be different. Increased staining for GFAP was accompanied by a significant increase in GS activity, and no effect was observed on the activity of the oligodendrocyte-specific enzyme. These results show that a sustained treatment with relatively low concentrations of TMT induced complex responses in aggregating brain cell cultures, in accord with findings *in vivo*, and that the characteristics of the glial responses to TMT are development-dependent.

DEMONSTRATION OF PERINEURONAL NETS IN THE AUDITORY CORTEX OF GERBILS USING CONFOCAL LASER SCANNING MICROSCOPY

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Perineuronal nets enwrapping cell bodies and proximal dendrites of a certain group of nonpyramidal cells are thought to represent a particular form of extracellular matrix molecules. In the cerebral cortex perineuronal nets were primarily observed on interneurons, most of which can be characterized by the coexistence of GABA with the intracellular Ca^{2+}-binding protein parvalbumin.
In the present study we investigated the perineuronal staining patterns of the plant lectin *Vicia villosa* agglutinin in the auditory cortex of gerbils (Meriones unguiculatus) by confocal laser scanning microscopy *(Leica TCS4D)*.
Double-immunofluorescence staining techniques were applied to analyze the coincidence with parvalbumin immunoreactive neurons.
The majority of VVA-covered neurons was located in layers III and IV and to a lesser extent in layers II and VI. Their somata were round or oval and could be classified as of the non-pyramidal type.VVA-staining revealed perineuronal nets that outlined the contours of nearly all parvalbumin positive cell bodies and very often the proximal portions of their dendrites. Usually, neurons which were enwrapped by perineuronal nets, displayed PV-IR of varying intensity throughout the perikarya and cell processes. However, some VVA-covered cells did not show PV-IR in their somata, although their corresponding proximal dendrites contained clearly visible PV-IR. Changes in the intracellular localization of the Ca^{2+} binding protein may occur as a consequence of differential electrical activity of the neurons. Whether such changes are reflected in alterations of the perineuronal net pattern is presently unknown but constitutes an interesting investigational concept.

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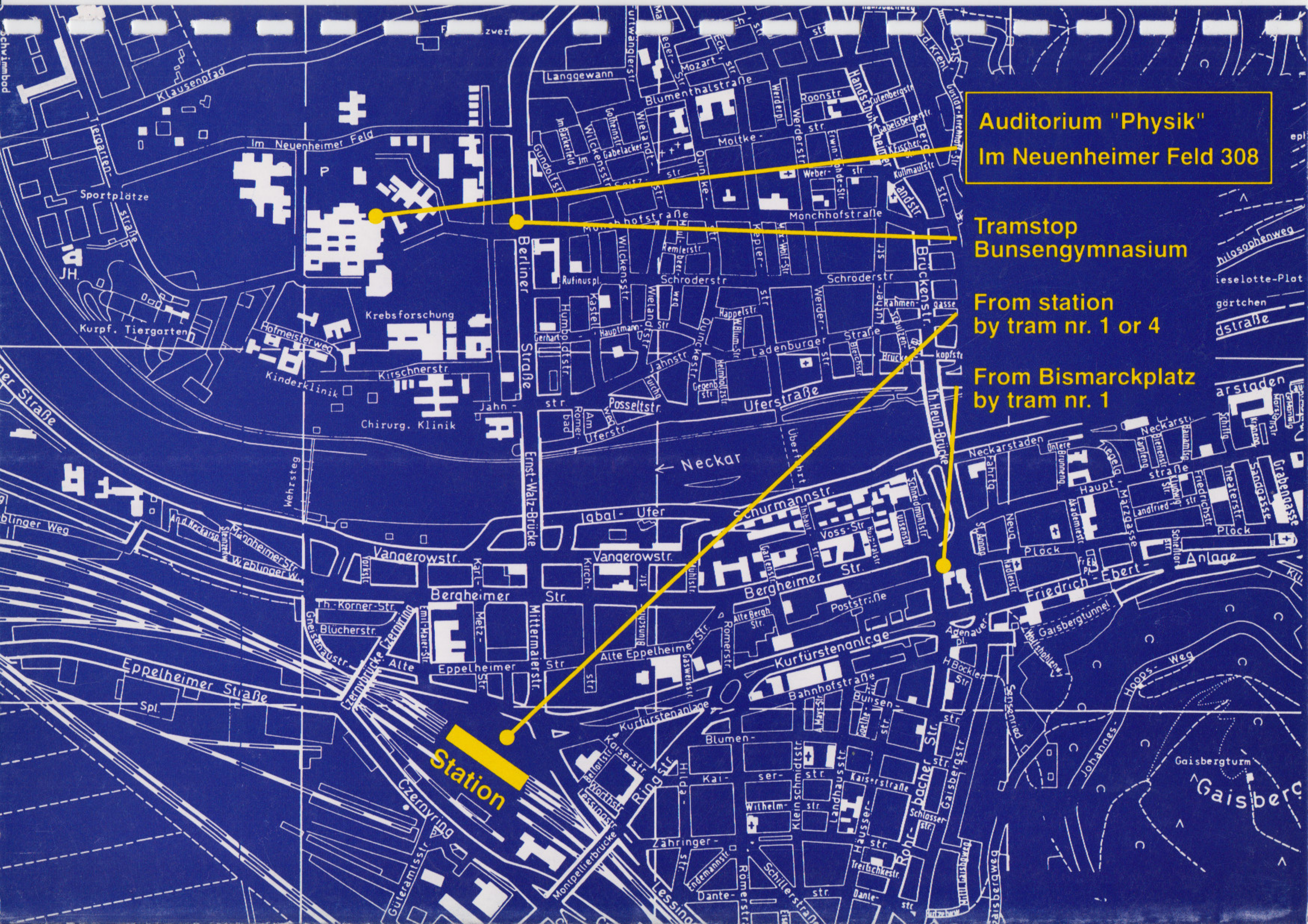
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