Neurosciences

1

CHANGES OF NUCLEAR 3,5,3' TRIIODOTHYRONINE RECEPTORS EXPRESSION IN DORSAL ROOT GANGLIA AND SCIATIC NERVE DURING DEVELOPMENT AND REGENERATION.

Barakat-Walter, I., and Duc, C. Institut d'Histologie et d'Embryologie, Faculté de médecine, rue du Bugnon 9, CH-1005 Lausanne.

Experimental and clinical data have shown that thyroid hormones play an essential role in assuring normal development and optimal function of the mammalian brain. The action of T3 hormones requires the presence of nuclear T3 receptors (NT_3R). The ontogenesis of NT_3R was studied in sensory neurons and Schwann cells of developing rat dorsal root ganglia and sciatic nerve. By using a specific monoclonal antibody raised to NT_3R, we have shown that the nuclei of both sensory neurons and Schwann cells expressed the NT_3R during the embryonic life. While the nuclei of sensory neurons continue to express the NT_3R in newborn and adult rats, the expression of NT_3R by Schwann cells vanishes gradually from birth and disappears at the end of the 2nd week. Sections of the sciatic nerve in adult rats caused a rapid reexpression of NT_3R by Schwann cells in segments adjacent to the lesion. These results suggest that thyroid hormones could be involved not only in peripheral nerve development but also in regeneration (SNF No. 31-26410-89).

2

INNERVATION OF SKIN AND MUSCLE OF CHICKENS BY SENSORY FIBRES EXPRESSING SUBSTANCE P (SP) OR CALBINDIN D-28k (CaBP)

Duc, C., and Barakat-Walter, I., Institut d'Histologie et d'Embryologie, Faculté de Médecine, rue du Bugnon 9, CH-1005 Lausanne

SP and CaBP are detected in two distinct subpopulations of primary sensory neurons in chickens. Peripheral projections of SP- or CaBP-immunoreactive neurons were studied in skin and skeletal muscle. In the skin, an extended network of thin SP-immunoreactive fibres gave rise to free endings underneath the epidermis and in the pulp of feathers. In contrast, CaBP-immunoreactive axons, restricted to particular skin areas, terminated by forming complex arborisations in a few connective papillae or apposed to most feather follicles. In muscle, thin SP-immunoreactive fibres formed free endings. On the opposite, large or thin CaBP-immunoreactive fibres innervated selectively neuromuscular spindles.
It is concluded that SP-immunoreactive sensory fibres are widely distributed in the skin and muscle of chickens, while CaPB-positive sensory fibres are restricted to specific targets or areas in these tissues. (SNF 31-26410.89)

3

CIRCADIAN RYTHMICITY OF SPONTANEOUS VASOACTIVE INTESTINAL PEPTIDE (VIP) RELEASE FROM MOUSE CEREBRAL CORTICAL SLICES

Zaerpour, B. and Magistretti, P.J. , Institut de Physiologie, Université de Lausanne, CH-1005 Lausanne

In rodent cerebral cortex, VIP is contained in bipolar interneurons and regulates energy metabolism within cortical columns. VIP can be released by depolarizing stimuli in a Ca^{2+}- and lipoxygenase-dependent manner. We have monitored the release of VIP, measured by RIA, during 24 hours. Mice were sacrificed at various time-intervals (every three hours) in the day/night cycle, their cortex was rapidly dissected, 250 x 250 µm slices were prepared and placed in a chamber superfused with oxygenated Krebs-Ringer bicarbonate buffer. After 90 min of washout, fractions were collected every two min for a 24 min period. Using this paradigm we have observed daily variations in the spontaneous release of VIP. Thus, VIP release reaches its maximum within 6 to 8 hours after the onset of light, declines gradually to a nadir six hours later and remains at these low levels during the dark period. Noradrenaline inhibits the spontaneous release of VIP following a similar pattern : inhibition is maximal (≈ 60 %) when basal VIP release reaches its peak and then gradually declines.

4

MOLECULAR CLONING OF DROSOPHILA MELANOGASTER CHOLINE ACETYLTRANSFERASE.

SALEM, N. and EDER-COLLI, L.*
Département de Pharmacologie, CMU, CH-1211 Genève.

We have previously shown that a hydrophilic and an amphiphilic form of choline acetyltransferase (ChAT) exist in *Drosophila*. These two forms have similar molecular weights on linear density gradients of sucrose. The amphiphilic enzyme requires the presence of detergent to be fully active.

We used molecular biological techniques aiming to understand how the amphiphilic form is attached to membranes. We screened *Drosophila* cDNA and genomic libraries with oligonucleotides synthesized on the basis of a non-full length *Drosophila* ChAT cDNA published by Itoh *et al.* (PNAS, 1986, 83, 4081). A 6.5 kb long ECORI genomic fragment was cloned which hybridized *in situ* to the the locus for the ChAT gene (91 B-D) on the *Drosophila* polythene chromosome 3R. A 1.9 kb long cDNA (non-full length) was also cloned which hybridized to this same locus and to the 6.5 kb long genomic fragment. The sequence of this cDNA clone was similar and showed identical restriction map to the one published by Itoh *et al.* Our cDNA also contained a 100 bp noncoding sequence which has consensus acceptor and donor sequences of a splice junction.

5

EXPRESSION OF GLIA-DERIVED NEXIN IN THE BACULOVIRUS SYSTEM

Brown-Luedi M., Rovelli G., Guidolin A. and Monard D., Friedrich Miescher-Institute, Postfach 2543, CH-4002 Basel

Glia-derived nexin (GDN) is a cell-secreted protease inhibitor which can promote neurite outgrowth in neuroblastoma cells and in some neuronal primary cultures. In vivo, GDN is overexpressed in nervous system structures undergoing degeneration or regeneration. GDN inhibits proteases such as urokinase, plasminogen activator and thrombin. Its thrombin inhibitory activity is greatly potentiated through association with heparin. Rat GDN has been expressed in yeast as an active but non-secreted protein. Here we report GDN expression in insect cells (sf9) with the Baculovirus expression vector system (BEVS). Rat GDN was expressed abundantly and the protein was secreted. Modified cDNA giving rise to GDN mutated in the putative heparin binding-site was also cloned. The recombinant proteins were purified and compared in regard to their structure and their interactions with thrombin and heparin.

6

EXPRESSION OF GLIA-DERIVED NEXIN AFTER IN VITRO LESION

Bleuel A., Meier R., Reinhard E. and Monard D.
Friedrich Miescher Institut, P.O. Box 2543, CH-4002 Basel.

Glia-derived Nexin (GDN) is a serine protease inhibitor promoting neurite outgrowth . In the peripheral nervous system, the expression of GDN is down regulated, but can be dramatically induced after lesion (Meier et al., Nature 343, 548-550 [1989]. In cultured dorsal root ganglia explants, it is possible to mimic the lesion in vitro. GDN, which is down regulated, can be induced following lesion of the processes emerging of the explants. Dotblotting quantification shows a maximal increase of 4-5 fold after 7 days. Double staining with specific antibodies demonstrates that the induction of GDN synthesis occurs only in Schwann cells and not in fibroblasts. The GDN increase is observed only in the Schwann cells distal to the site of injury, where neuronal contact has been lost as consequence of nerve degeneration. These results show that the effects seen in vivo can be replicated in vitro. They suggest the existence of a neuronal component which represses the expression of GDN in Schwann cells.

7

MYELIN/OLIGODENDROCYTE GLYCOPROTEIN (MOG): LOCALIZATION, DEVELOPMENTAL EXPRESSION AND ISOLATION.

Patrick Amiguet, Minnetta V. Gardinier and Jean-Marie Matthieu, Service de Pédiatrie, Laboratoire de Neurochimie, CHUV, 1011 Lausanne.

Myelin is a multilamellar membranous structure that surrounds axons of the peripheral and central nervous system (CNS). Myelin/oligodendrocyte glycoprotein (MOG) was first identified by a monoclonal antibody. Recent studies showed that MOG plays an important role as a target for antibody-induced demyelination. Immunocytochemical studies have shown that MOG is specifically expressed in the CNS on the surface of myelin and oligodendrocyte processes. Immunoblot analyses revealed that MOG consists of two bands with apparent molecular masses (Mr) of 28 and 26 kDa. After deglycosylation only one band of Mr 25 kDa was identified, indicating that MOG presents different glycosylation levels. MOG concentrations increased during the most active phase of myelin deposition. MOG is almost absent from jimpy mice which possess a proteolipid protein gene defect and are nearly devoid of CNS myelin. In mld mutant mice, which are affected by a severe deficit of myelin basic protein (MBP) synthesis, MOG was present at drastically reduced levels. In mld mutants, at 85 days of age, MBP concentrations increase and myelin is better compacted. At this age, MOG concentrations increased and reached 70% of controls. These results suggest that MOG plays a key role in the maintenance or completion of the myelin sheath. In order to understand its structure and function, we purified MOG using conventional biochemical techniques. A sequence of 26 amino acids was obtained and a cDNA library was screened with three oligonucleotides derived from this sequence .

8

STRUCTURAL ASSESSMENT OF *N*-LINKED OLIGOSACCHARIDES OF THE TWO MAJOR HUMAN MYELIN GLYCOPROTEINS: MAG AND P_o.

Burger D., Perruisseau G. and Steck A.J.
Laboratoire de Neurobiologie, 1011 CHUV/Lausanne

Myelin-associated glycoprotein (MAG) and P_o are both myelin glycoproteins that belong to the immunoglobulin superfamily and express the L2/HNK-1 carbohydrate epitope that is found on numerous neural cell adhesion molecules. Since carbohydrates are thought to play a role in the adhesion/recognition function of these myelin glycoproteins, the *N*-linked oligosaccharide structures of MAG and P_o were characterized by serial lectin affinity chromatography (SLAC). Isolated human dMAG and P_o were reduced, carboxymethylated and then extensively digested with thermolysin. The glycopeptides were [^{14}C]-labelled and submitted to SLAC analysis on ConA, RCA_I, E_4-PHA, PSA, and L_4-PHA. The results show that both MAG and P_o display a high degree of heterogeneity in their oligosaccharide structures that were mainly of the fucosylated bisected complex type. Furthermore, the structures bearing the L2/HNK-1 epitope were identified.

9

ANTI-IDIOTYPIC ANTIBODY-PSEUDOMONAS EXOTOXIN A CONJUGATE AGAINST HNK-1 SECRETING CELLS.

Bourdenet S., Perruisseau G., Burger D. and Steck A.J.;
Laboratoire de Neurobiologie, 1011 CHUV/Lausanne.

In demyelinating neuropathy associated with monoclonal IgM antibodies to the myelin associated glycoprotein (MAG), current evidence suggest a causative role of autoantibodies in the disease. We have investigated the use of an immunotoxin composed of an anti-idiotypic antibody linked to a potent bacterial toxin (Pseudomonas exotoxin A (PE)), to eradicate B cells producing monoclonal anti-MAG antibodies. The monoclonal mouse IgM HNK-1 is also directed against MAG. We have raised a monoclonal anti-idiotypic antibody (G6D9) directed against HNK-1 displaying high affinity and speficity for HNK-1 bearing cells. A G6D9-PE conjugate was obtained by coupling the antibody to the toxin via a disulfide bridge. In cell culture, the measure of inhibition of protein synthesis shows that the immunotoxin displays higher cytotoxicity for the HNK-1 cells as compared to the control cells. Research is in progress to increase the specificity of the conjugate without reducing its potency.

10

TRIIODOTHYRONINE HORMONE (T3) STIMULATES EXPRESSION OF MYELIN PROTEIN GENES IN AGGREGATING BRAIN CELL CULTURES.

Tosic,M., Torch,S., Comte,V., Dolivo,M., Honegger,P. and Matthieu,J.-M.
Service de Pédiatrie, Laboratoire de Neurochimie, CHUV, 1011 Lausanne.

Triiodothyronine (T3) plays an important role in development of the nervous system. Previous experiments with the aggregating brain cell cultures showed that T3 stimulates myelination affecting differentiation of the oligodendroglial cell. Increased activity of 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) as well as higher concentrations of myelin basic protein (MBP) were observed. In this study we measured the expression of these two myelin protein genes at the mRNA level. Overall concentrations of both MBP and CNP specific mRNAs were higher in the presence of T3. Since it is known that T3 stimulates transcription of various genes, we assayed transcription in nuclei isolated from cultures grown in the presence or absence of T3. The transcription rate of the CNP gene was significantly higher in cultures treated with T3. No difference was found for the MBP gene. However, when we blocked transcription adding actinomycine D to the cultures, we could detect differences in degradation rates of specific mRNAs. MBP mRNA showed higher stability in the presence of T3. The half-life of MBP mRNA was reduced by about five hours in cultures deprived of T3. Furthermore, in vitro translation experiments showed that T3 stimulates specifically MBP translation. Our results show that T3 has diverse stimulating effects on specific genes, increasing rate of transcription, stabilizing mRNA and/or stimulating translation.

11

C6 RAT GLIOBLASTOMA CELLS USE A SPECIFIC PROTEOLYTIC MECHANISM TO OVERCOME THE INHIBITORY SUBSTRATE EFFECT OF CNS WHITE MATTER FOR INVASIVE CELL MIGRATION

V.Amberger; M.E.Schwab and P.A.Paganetti;
Brain Research Institute, University of Zurich, August-Forel-Str. 1, CH-8029 Zurich.

The C6 glioblastoma cells are a good model to study infiltrative properties of CNS tumor cells. We showed that they are able to overcome the inhibitory substrate properties for cell migration expressed on oligodendrocytes and CNS white matter. A metalloproteolytic activity associated with C6 cell membranes enables the cells to inactivate these inhibitors (Paganetti et al.; J.Cell Biol. 107:2281-2291,1988). A wide range of known enzyme blockers and newly developed peptides were used to determine the specificity of this protease. A recently developed substrate assay allowed us to determine the specific localization of the protease to the plasma membrane and its pH-optimum (5,5 - 6,5). Solubilization of the enzyme was obtained with detergents.

12

REGENERATION OF LESIONED FIBER TRACTS IN THE HIPPOCAMPUS AND SPINAL CORD OF RATS

D. Cadelli, L. Schnell, and M.E. Schwab, Brain Research Institute University of Zurich, August-Forel-Str.1, CH-8029 Zurich

The septo-hippocampal connection (fimbria-fornix) or a majority of the spinal cord fiber tracts were interrupted surgically in 2-6 week old rats. An antibody neutralizing the myelin-associated neurite growth inhibitors was applied by implanting AB-secreting hybridoma cells as tumors or in millipore filter "ravioli". In the hippocampus the large lesion was bridged by an implant of extracellular matrix soaked with NGF. Regenerating AchE-positive septal fibers readily crossed the bridge and entered the hippocampus. In the controls, their growth subsided within 0.2-1 mm ($\bar{x}=0.62 \pm 0.01$, $n=5$). In contrast, regeneration distances of 1-4 mm were observed in the IN-1 treated hippocampi ($\bar{x}=1.8 \pm 0.15$, $n=15$) within 2-5 weeks after the lesion. - In the spinal cord, highly significant enhancement of regeneration was observed for the corticospinal tract (maximal distances: 8-15 mm). At the lesion site regenerating fibers invariably grew through remaining spinal cord tissue rather than across the implanted bridges consisting of a variety of materials. - These results shows that oligodendrocyte-associated neurite growth inhibitors play a major role in preventing fiber regeneration in the hippocampus and spinal cord.

13

STABILIZATION OF OPTIC AXON NUMBERS BY OLIGODENDROCYTES

R.J. Colello and M.E. Schwab., Brain Research Institute, University of Zürich, August-Forel-Str. 1, CH-8029 Zürich

Differentiated oligodendrocytes start to express neurite growth inhibitors at a time when these cells are involved in the myelination of recently formed fibre pathways. The presence of these inhibitor proteins, concurrent with the completion of fibre outgrowth and the establishment of terminal fields in the target, suggests that they may have a role in stabilizing fibre projections.

To test this hypothesis, the retinofugal pathway of rats was X-irradiated at postnatal days 0, 2, and 4 to remove oligodendrocyte percursor cells. EM analysis of irradiated optic nerves at P15 shows that the absence of oligodendrocytes and myelin was complete. Preliminary axon counts showed that the total fibre number in the optic nerve of irradiated animals was 25% higher than that of normals. Further, counts at three locations along the length of an irradiated optic nerve showed a fluctuation of approx. 27%, while axon numbers in normals fluctuated at most 5%.

These results suggest that, in the absence of myelination, optic fibres are able to form sprouts. This hints at oligodendrocytes having a role in preventing sprouting and stabilizing the number of fibres in a pathway during development.

14

STUDY OF THE IMFLAMMATORY RESPONSE IN THE ADULT MOUSE BRAIN AND SPINAL CORD AFTER GRAFTING OF EMBRYONIC MOUSE MOTONEURONS

B. Demierre*, P. Ruiz-Flandes and A.C. Kato, *Dept. Pharmacology, CMU, Geneva, Switzerland; Dept. Neurosurgery, Univ. Hospital of Geneva, Switzerland**

Grafted embryonic motoneurons survive better in the brain as compared to the spinal cord and this difference appears to be related to the trauma caused by the grafting procedure. In the brain, there was almost no inflammatory response or reactive gliosis at the lesion site following 4 to 10 weeks of grafting. In contrast, there was approximately a 50-fold increase in the area of the inflammatory response in the spinal cord and this was accompanied by an intense reactive gliosis and by a degeneration of the host motoneurons. Many more macrophages accumulated in the lesion site of the spinal cord as compared to the brain. These differences may result either from the type and the quantity of the immune cells which infiltrate the lesion site or from a variability of the astrocyte populations in the CNS.

15

A SIMPLE METHOD FOR PREPARING ORGANOTYPIC CULTURES OF NERVOUS TISSUE.

Stoppini, L., Buchs, P.-A. and Muller, D. Département de Pharmacologie, Centre Médical Universitaire, 1211 Geneva 4.

A method is described which allows to prepare and maintain slices of nervous tissue in culture for several weeks. Most experiments were carried out using hippocampal slices. 450-500 μm thick slices of 2-23 day old neonates were prepared using a tissue chopper. They were placed on a poreous and translucid membrane at the interface between a regular culture medium (MEM + 25% horse serum) and an atmosphere containing 5% CO_2. No plasma clot was necessary to attach the slices on the membrane and no rolling action was used. In those conditions slices survive for several weeks, keep their organotypic organization and tend within a few days to flaten into a mono- or bilayer. However they always remain about 100-150 μm thick. The results of extra- and intracellular horse radish peroxydase injections as well as extra- and intracellular recordings show that the typical features of hippocampal pyramidal neurons are preserved. During the first days in culture the size of synaptic responses considerably increases and growth cones can be visualized by HRP labellings. These observations suggest that an important sprouting response occurs in the organotypic cultures during that period of time. This technique may represent an interesting alternative to roller tube cultures for studies of the developmental changes occurring during the first days or weeks in culture. Work supported by FNRS 3.173.0.88.

16

GLYCOGEN METABOLISM IN PRIMARY ASTROCYTE CULTURES

Sorg, O. and Magistretti, P.J. Institut de Physiologie, Université de Lausanne, 1005 Lausanne.

Glycogen is the single largest energy reserve in the brain. Histological studies have revealed that in CNS, glycogen is predominantly localized in astrocytes. In recent years, receptors for various neurotransmitters have been demonstrated in primary astrocyte cultures. Our interest was focused on the potential glycogenolytic effect of neurotransmitters in primary cultures of astrocytes prepared from neonatal mouse hemispheres. Vasoactive Intestinal Peptide (VIP) and two related peptides, PHI and secretin, induced the hydrolysis of glycogen, as well as the neurotransmitters noradrenaline (NA), dopamine and adenosine. Pharmacological studies showed that the effect of NA was mediated by β-adrenergic receptors. The second messenger involved is likely to be cAMP, since dBcAMP and forskolin mimicked the effect of the neurotransmitters. Pretreatment with dibutyryl cAMP promoted a dramatic increase in glycogen synthesis, as well as glycogen breakdown. These observations further stress the notion of a neuron-glia functional partnership aimed at maintaining local energy metabolism homeostasis.

17

MODELS FOR STUDYING METABOLISM AND FUNCTION OF GLIAL CELLS

Tsacopoulos, M. and Poitry-Yamate, C., Experimental Ophthalmology Laboratory, HCUG, CH-1211 Genève 4

An essential function of glial cells is to contribute to the satisfactory working of neurons. We have explored glial function in the isolated retina of the honeybee drone and purified Müller glia from the juvenile guinea pig. In the first, the glia contain almost no mitochondria but large quantities of glycogen. Phoreceptors, on the other hand are mitochondria rich and have a very active aerobic energy metabolism. Glia transform glucose and supply continuously a metabolic substrate to the photoreceptors. Glial carbohydrate metabolism is modified when photoreceptors are stimulated. However, since no direct response of glia to light has been detected a signal passes from photoreceptors to glia. Measurements with ion-selective microelectrodes showed large ionic movements elicited by photostimulation. Müller glia *in situ* in the juvenile guinea pig retina contribute an important role in the overall metabolism of glucose. In our second model we have isolated Müller glia and have shown that they take up and phosphorylate [^{3}H]-2-deoxyglucose. They are metabolically active as this phosphorylation is strongly inhibited by iodoacetate.

18

AXON-GLIAL CELL INTERACTIONS IN RABBIT VAGUS NERVE.

Brunet P., Volet B. and Jirounek P.
Département de Pharmacologie, C.M.U., 1211-Genève 4, Suisse.

Interactions between axons and Schwann cells were studied in rabbit vagus nerves by the sucrose-gap method. Action potential (AP), and the hyperpolarizing and depolarizing afterpotentials (HAP and DAP respectively) were recorded in normal nerves and in nerves submitted to axonal degeneration. After 3, 6 and 10 days of Wallerian degeneration, the AP decreased by 27.5, 77.7 and 100% respectively. During the degeneration, the HAP disappeared in parallel to the AP, while the DAP persisted or even increased. Interestingly, the same stimulus which elicited an AP in the normal nerve, evoked in the 15 days-degenerated nerve a slow depolarizing wave (SDW), lasting for several minutes and slowly propagating. Axonal regeneration was induced by a second operation and after 1, 2 and 3 months, the AP reapeared and reached about 28.3, 36.4 and 81.3% respectively of the AP in the controlateral nerves (used as controls). Concomitantly the SDW vanished and turned into the ADP (53, 33 and 24% respectively of the SDW in the 15 days-degenerated nerves). These results strongly support the idea that both the SDW and the ADP originate from the Schwann cells, which are still present in the degenerated nerve. Moreover, they indicate that glial cells in the nerves are not completely silent and that their numerous, recently identified voltage-dependent ionic channels, could play an important role with respect to the mechanisms involved in the axono-glial interactions.

19

OPTICAL RECORDING OF POSTSYNAPTIC POTENTIALS IN MOTONEURONS IN ORGANOTYPIC RAT SPINAL CORD SLICE COCULTURES

M.G. Rioult and H.-R. Lüscher, Dept. of Physiology, University of Bern, CH-3012 Bern, Switzerland.

Optical recording were used to study evoked electrical activity in dendrites and the soma of motoneurons (MN) in organotypic cocultures of embryonic rat spinal cord with attached dorsal root ganglion and skeletal muscle.
The cultures grown for 14 days in vivo, were stained with voltage-sensitive dyes (e.g. RH-237, di-4-ANEPPS) at concentrations of 5-20 μM for 5-20 minutes. Changes in fluorescence intensity were recorded simultaneously by photodiodes (PD) of a 12x12 element PD array mounted in the image plane of an inverted microscope. 40x/1.3 n.a. glycerine immersion objectives provided a spatial resolution of 14 μmx14 μm/PD. The signal from each PD was amplified and digitized at a maximal rate of 3.0 kHz/PD for up to 330 msec.
The electrical activity in MN was evoked by extracellular focal stimulation of the ipsi- or contralateral dorsal horn and could be recorded by the PDs covering the cell body and the dendrites of the MN without signal averaging.
The recording showed that the activity pattern in the dendrites as well as in the cell body were dependent on the stimulation site in the spinal cord. Moreover, in a serie of subsequent stimulations under identical stimulation and recording conditions the dendritic activity pattern was subject to variations ranging from slight changes in the shape of the recorded potentials up to a failure of the potential to appear in certain dendrites.
(Supported by SNF No. 3.625-0.85)

20

SYNTHESIS OF PGE_2 AND OF AN UNUSUAL PROSTAGLANDIN BY CHICK SPINAL CORD MENINGES

Vesin M.-F.

Institut d'Histologie et d'Embryologie, Université de Lausanne, rue du Bugnon 9, 1005 Lausanne

Metabolism of $[1\text{-}^{14}C]$ arachidonate was investigated in spinal meninges homogenates. After lipid extraction, separation on TLC revealed the synthesis of large amounts of PGE_2 as well as a less polar product referred to as compound Y. Formation of compound Y is inhibited by indomethacin and enhanced by esculetin, hence compound Y generation is mediated through the cyclooxygenase pathway and is not due to degradation of PGE_2. Furthermore compound Y possesses 1) a prostanoid structure since it is converted into PGF_2 (α,β) after strong reduction with $NaBH_4$; 2) an hydroperoxyde group which is cleaved by mild reduction with $SnCl_2$ or GSH-hemin leading to PGE_2.
It is inferred that compound Y corresponds to 15-hydroperoxy PGE_2 (SNF No. 3397.86).

21

BIOSYNTHESIS OF PGD_2 AND LOCALIZATION OF PGD_2 SYNTHETASE IN CHICK SPINAL CORD

Billotte C., Vesin M.-F., and Droz B., Institut d'Histologie et d'Embryologie, rue du Bugnon 9, Université de Lausanne, CH-1005 Lausanne

In chick spinal cord homogenates $[1\text{-}^{14}C]$ arachidonate was metabolized through cyclooxygenase pathway into various prostaglandins (PG) including PGD_2, PGE_2 and $PGF_{2\alpha}$. In an attempt to localize the sites of PGD_2 synthesis, spinal cord sections were incubated with polyclonal antibodies raised to rat brain PGD_2 isomerase. After control of the specificity, the density of the immunostaining was:
1) moderate in the perikaryon of particular motoneurons;
2) extremely high in nerve endings encompassing motoneuron cell bodies and dendrites.

Since PGs act at the vicinity of their sites of synthesis, it is therefore suggested that PGD_2 could play a role in the regulation of motor functions (SNF No. 3397.86).

22

MAP2 AND TAU PROTEINS IN DEVELOPING CAT CEREBRAL CORTEX AND CORPUS CALLOSUM

Riederer B.M., and Innocenti G.M.
Institut d'Anatomie, Rue du Bugnon 9,1005 Lausanne

We have studied two groups of microtubule-associated proteins (MAPs), MAP2 and tau's, during the postnatal development of cat cortex and corpus callosum, using well characterized monoclonal antibodies for MAP2 (AP14 and C) and for Tau (TAU-1). During the first five weeks of postnatal development both MAPs change in their molecular composition from juvenile type to adult type proteins. They differ in their subcellular distribution, in that MAP2 isoforms MAP2b and MAP2c, and phosphorylated Tau proteins are especially present during establishment of dendritic processes in pyramidal cells, while other Tau forms were found mostly in axons. Phosphorylated isoforms of MAP2 and Tau proteins are prominent during early stages of development. Biochemical experiments indicate that phosphorylation mediates the binding of MAPs to other structural components. These data consolidate our previous findings on other cytoskeletal proteins: MAP5 and the large neurofilament subunit, and support the view that changes in phosphorylation may be involved in growth and stabilization of dendrites and axons. In addition, we have found MAP2 containing neurons in the juvenile corpus callosum, using AP14 and immunofluorescent double-labelling with neuron specific enolase, GABA and GFAP polyclonal antibodies. Only in the frontal part of adult corpus callosum some neurons persisted. Connections of these neurons and their possible role in early guidance of callosal axons remains to be clarified.
This work was in parts supported by Sandoz Foundation, the University of Lausanne and SNF projects No. 31-26624.89 and 3.359-0.86.

23

EXPRESSION OF NEURON-SPECIFIC MOLECULAR LABELS ON GROWTH CONES AND DEVELOPING SYNAPTIC TERMINALS IN THE GRASSHOPPER EMBRYO.

Meier, T., Friedli, M., Reichert, H., Dèpartement de Biologie Animale, Université de Genève, CH-1211 Genève.

Neuron-specific molecular labels may be important for establishing the correct pattern of synaptic connections during neuronal development. We have discovered such cell-specific molecular labels on the growth cones and developing synaptic terminals of identified neurons in the grasshopper embryo. One of these molecular labels is expressed in two identified interneurons which have their cell bodies and dendritic arbors in the brain and project their axons to the ventral ganglia. During axonogenesis, the label is found on the axonal growth cones of these neurons. Later during synaptogenesis, the label becomes concentrated on the membrane of the presynaptic boutons of these neurons. This molecular label is not expressed in any other of the millions of neurons in the nervous system, nor in any other cell in the developing embryo. These findings suggest the existence of a molecular labeling system that is remarkably specific for individual cells and may be important for establishing precise synaptic connectivity. The isolation and characterization of these molecular labels are now in progress. (Supported by the FNS).

24

CHARACTERIZATION OF GENE PRODUCTS EXPRESSED DURING SYNAPTOGENESIS IN THE CHICK RETINA.

M.-C. Lebeau, G. Alvarez-Bolado, O. Braissant, W. Wahli, and S. Catsicas. Institut de Biologie animale. Université de Lausanne.

During synaptogenesis, cell-cell interactions between neurons and their targets are thought to induce the expression of specific sets of genes. These genes might encode structural components of the synapse as well as proteins involved in neuronal differentiation. In the chick retina, synaptogenesis begins at embryonic day 11 (E11) and the density of synapses reaches adult levels by E15. Therefore, in order to characterize genes regulated during synaptogenesis, we have constructed 2 cDNA libraries from retina mRNA extracted at E9 and E15. We have then developed a new sensitive method of subtractive hybridization, based on DNA-DNA competitive hybridization with PCR amplified libraries. We have isolated several clones corresponding to mRNAs that show developmental regulation during synaptogenesis and are nervous system specific. One such clone was found to encode SNAP-25, a nerve terminal protein specific for subtypes of synapses.

25

DIFFERENTIAL EXPRESSION OF PRESYNAPTIC PROTEINS DURING POSTNATAL DEVELOPMENT OF CAT VISUAL CORTEX.

G. Alvarez-Bolado, G.M. Innocenti*, W. Wahli, and S. Catsicas. Institut de Biologie animale and Institut d'Anatomie*, Université de Lausanne.

Recent evidence suggests that the differential expression of presynaptic proteins contributes to the formation of synapses with different molecular phenotypes. As a first step in evaluating the factors responsible for this selective expression, we have studied the distribution and developmental expression of SNAP-25, synapsin I and synaptophysin in the cat visual cortex. Each protein had a distinct spatio-temporal pattern of expression, as shown by immunocytochemistry and, in addition, the three proteins seemed to undergo developmentally regulated changes in subcellular localization. At P1, SNAP-25 was found only in axons of layer I, synapsin I was found throughout the cortex in the neuropil as well as cell bodies and synaptophysin was localized mainly in presumptive nerve terminals. In adults, all three proteins were mainly localized within boutons, but showed clear differences in expression across cortical thickness.

26

DETECTION OF CELL TYPE SPECIFIC ANTIGENS IN REAGGREGATE CULTURES OF CHICK EMBRYONIC NEURONAL RETINA

Shah-Derler, B. and Bruinink, A.
Inst. für Toxikologie der ETH und Univ. Zürich,
CH-8603 Schwerzenbach

In order to study the in vitro development of the neuronal retina and mechanisms causing drug-induced retinopathy, serum-free, rotation-mediated aggregating cell cultures from the embryonic chick neuronal retina were prepared. Immunohistochemical studies on reaggregate semi-thin sections and sandwich ELISA using reaggregate homogenates were carried out on Day 14 of culture. The ELISA methodology was optimized for several differentiation markers, including PNA-lectin, which binds to ß-galactose-galactosamine membrane protein residues, and monoclonal antibodies against tyrosine hydroxylase, MAP5 and neurofilament 68KD. Maximum binding of the antigens was reached at a protein concentration of 0.1 mg/ml for all markers. By changing various parameters, e.g. coating temperature and duration, fixation, type of buffer, addition of BSA, different detergents, etc., specific and non specific binding were strongly affected.

27

IN VITRO DIFFERENTIATION OF EMBRYONIC CHICK BRAIN CELLS: DEVELOPMENT OF A NEUROTERATOLOGY TEST SYSTEM

Reinhardt, C.A., Bienz, A., Romano-Diethelm, T. and Wyle-Gyurech, G.G.; Cell Lab SIAT, Behavioral Biology, ETH-Center, CH-8092 Zurich, Switzerland.

In order to develop a model for potential neurotoxicity and teratogenicity, chick brain cells (embryonic day 7, ED7) were mechanically dissociated and cultured up to several months. Differentiation of nerve and glial cells (in petridishes, monolayers; and suspension cultures as reaggregates) were monitored with monoclonal antibodies against 68kD neurofilament protein (anti-NF) glial fibrillary acidic protein (anti-GFAP) and tyrosine hydroxylase as well as by monitoring the protein synthesis pattern by 2D-gel electrophoresis.

Anti-NF stains neurons in vitro as they differentiate morphologically. In reaggregates a stable differentiation of nerve cells could be observed by intensive anti-NF staining for as long as 3 wk in culture. 5 wk old cultures still showed substantial staining. The expression of NF in nerve cells is proposed to be used as a sensitive cytotoxicity endpoint whereas GFAP expression can serve as an endpoint for monitoring differentiation of neural tissue (Wyle & Reinhardt, *Toxicol. In Vitro*, in press).

Monitoring of functional parameters of the dopaminergic system (such as activity of tyrosine hydroxylase) and further general endpoints (protein metabolism, cytotoxicity) wil be combined to evaluate the in vitro toxic potential of known neuroteratogens such as psychoactive drugs and analgetica.

28

N-ACETYL-L-ASPARTATE IS A MAJOR SOURCE FOR ACETYL GROUPS FOR LIPID SYNTHESIS DURING RAT BRAIN DEVELOPMENT

R. Burri, N. Herschkowitz, Department of Pediatrics, University of Bern

To investigate the possible function of N-acetyl-L-aspartate (NAA) as a lipid precursor in the CNS [^{14}C]-NAA was injected intracerebrally into 8, 15 and 22-day-old rats. During an incubation period of 4 hours radioactive acetyl groups from NAA were incorporated into the lipid fraction in amounts of 42.9-65.7% of total recovered radioactivity, *increasing with the age of the rats (Tab 1). Only 7.2 to 9.4% were* incorporated into the protein fraction. In contrast, in control rats incorporation of [^{14}C]-acetate (AcA) into the lipid fraction was not age-dependent with values from 50 to 56%, while 27.0 to 18.1% were incorporated into the protein fraction, the amounts decreasing with age.

Tab 1: *Incorporation from NAA*	Radioactivity in the lipid fraction (%)	Radioactivity in the protein fraction (%)
8 days	42.88 ± 9.80	7.20 ± 0.91
15 days	57.95 ± 10.38	8.73 ± 0.93
22 days	65.66 ± 5.96	9.38 ± 2.41
Incorporation in % of total radioactivity recovered (mean ± SD, n = 4)		

Taking into account that in vivo NAA concentration in the brain is about 10 times higher than the AcA concentration, NAA is clearly the more efficient precursor for lipid synthesis than AcA. We conclude that NAA is a major source of acetate groups for lipid synthesis during rat brain development. (supported by SNSF grant 3.156-0.88)

29

AN IMMUNOCYTOCHEMICAL COMPARISON OF THE ANGIOTENSIN AND VASOPRESSIN HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEMS IN NORMOTENSIVE AND HYPERTENSIVE RATS

Imboden,H. and Felix,D., Universität Bern, Abteilung Neurobiologie, Erlachstrasse 9a, 3012 Bern

Our aim was to show whether angiotensin (ANG) II/III and vasopressin (VP) coexist in the hypothalamo-neurohypophysial pathway. We used 8-week old male rats not treated with colchicine and examined a series of coronal, horizontal and sagittal sections. Arching fibre tracts are formed mainly by projections emanating from cell bodies in the paraventricular nucleus, the accessory magnocellular nuclei, the supraoptic nucleus and the retrochiasmatic part of the supraoptic nucleus. The majority extend as far as the median eminence and the neurohypophysis, where major terminal fields exist. However, the staining pattern is different in the suprachiasmatic nucleus and the hypophysis. We then compared the ANG and VP systems in normotensive and hypertensive rats. Although we were not able to show any differences between the strains, our results clearly show that ANG and VP coexist in neurones and fibres of the hypothalamo-neurohypophysial system.
Supported by SNF grant 31-29986.90.

30

VISUALIZATION AND QUANTIFICATION OF QUINOLINIC ACID LESIONS IN RAT BRAIN BY MAGNETIC RESONANCE IMAGING (MRI)

Sauter A. and Rudin M., SANDOZ-PHARMA Ltd, CH-4000 Basel

A current hypothesis links neuroexcitatory properties, i.e. activation of the NMDA receptor complex, of excitatory amino acids (EAA) to certain neurodegenerative disorders, such as ischemic stroke, Alzheimer's and Parkinson's disease. Axon-sparing lesions are induced in rat brain by local injection, e.g. into the striatum, of quinolinic acid (QA), an endogenous EAA. Such neurotoxic effects can be prevented by drugs interacting either directly with the NMDA channel, such as MK-801, with the NMDA recognition site, such as CPP-ene, or with the allosteric glycin site, such as 7-Cl-kynurenic acid. The extent of the lesion usually is quantified 7 days later by determination of CAT and GAD activities, two markers of striatal interneurons. Here we report that, using in-vivo MRI, such lesions can be detected and quantified reliably already after one day. We found that the lesion size, as determined by MRI, increases linearly with the dose of QA (100, 200, 400 nmole) and that identical drug effects, as reported using enzyme assays, are found. A good correlation between lesion size and changes in K^+ and Na^+ levels, determined post-mortem, further suggests that QA causes edema within 24 hours, detectable by MRI and ultimately leading to degeneration of interneurons, evident after 7 days.

31

THE EFFECTS OF PRENATAL STRESS ON POSTNATAL COGNITIVE BEHAVIOR IN RATS

Szuran, T., Zimmermann, E., Bättig, K., Welzl, H., ETH, Lab. Behavioral Biology, CH-8092 Zürich

Prenatal stress in rats has been shown to especially influence sex specific behaviors of the offspring. To further investigate the role of prenatal stress, pregnant rats were subjected to repeated immobilization stress during gestational Days 15-19. The behavior of their offspring was compared with that of the offspring of unstressed rats. The offspring were tested for negative geotaxis and for their nest-odor-preference on postnatal Days 6 and 10, respectively. At the age of 4 months, the offspring were tested for their learning and memory abilities in the Morris-water-maze. Vestibular stimulation induced a faster reaction of prenatally stressed pups in the negative geotaxis test. Prenatally stressed pups showed a decreased nest-odor-preference compared to the unstressed controls. Further, in the water maze the prenatally stressed offspring spent more time searching for an escape platform in the quadrant in which the platform had been during training trials than did the prenatally unstressed rats.

32

DOES REFLEX ENHANCEMENT BY POST-TETANIC POTENTIATION AND BY COOLING ACT VIA THE SAME MECHANISM IN THE FROG SPINAL CORD?

M.-S. Rioult-Pedotti and H.P. Clamann, Dept. of Physiology, University of Bern, CH-3012 Bern, Switzerland.

PTP may increase the reliability of AP propagation through branch points and thus increase the number of synaptic boutons activated. Low temperature increases action potential (AP) duration which improves their propagation across axon branches. The mechanisms underlying the enhanced synaptic efficacy following tetanic stimulation are not well understood, however.

Isolated, hemisected frog spinal cords were placed into a superfusion chamber and heated or cooled with Peltier elements. The lumbar dorsal (DR) and ventral roots (VR) were drawn into suction electrodes. DR's were stimulated with single shocks (1/s) before and after tetanic trains of 500/s for 3s. Reflex responses were recorded in VR's of the same segment at constant temperatures between 4.4° and 22°.

Reflex responses, as judged by their peak amplitude or area, elicited by a single DR stimulus were much larger in the cold than in the warm cord where often only spontaneous activity was seen. PTP always augmented the monosynaptic or shorter-latency response at the expense of a longer-latency response. Reflex latency increased with higher temperatures in the unpotentiated cord. After tetanic stimulation peak amplitude and area were affected similarly: at low temperature (4.4°-10°) a tetanus produced reflex depression while in the warm cord (18°-20°) the reflex was potentiated. We suggest that temperature and PTP produce changes in synaptic efficacy by operating on the same mechanism or mechanisms. (Supported by Sandoz-Stiftung, SNF No. 31-27973.89.)

33

RESPONSES OF MONKEY DOPAMINE NEURONS TO INCENTIVE STIMULI DURING LEARNING

W. Schultz, T. Ljungberg and P. Apicella. Institut de Physiologie de l'Université,1700 Fribourg

We investigated the motivational nature of responses of dopamine (DA) neurons of midbrain areas A8, A9 and A10 during learning of behavioral tasks in 2 Macaca fascicularis monkeys. DA neurons responded to exactly those stimuli that were the key signals for learning the task contingencies during the different epochs of learning. These were (i) novel stimuli eliciting exploratory behavior, (ii) primary liquid reward while the behavioral reaction was being established, and (iii) the conditioned stimulus leading to acquisition of reward. According to motivation theory, these three stimuli have incentive value for the behavior of the animal because they elicit the behavior and at the same time guide the subject toward the goal of behavior, i.e. obtaining reward. In line with this interpretation, reduction in incentive stimulus properties after stereotyped, automated task performance following extensive overtraining considerably reduced neuronal responsiveness. These data demonstrate an involvement of DA neurons in motivational processes underlying a wide variety of behavior.

34

DOES PUPIL-SIZE PLAY A ROLE IN THE AETIOLOGY OF WINTER DEPRESSION?

V. Lacoste and W. Pöldinger, Psychiatric University Clinic, CH-4025 Basel, Switzerland

Since Lewy 1982 first described the beneficial effect of phototherapy on winter depression, a form of seasonal affective disorder (SAD), a variety of mechanisms underlying this disturbance and its treatment have been proposed. None of them however, implicated pupil-size, although - by regulating the amount of light entering into the eye - it plays an essential role in short- and longterm adaptation to changing environmental illumination.

Studying physiological variability of the dark adapted pupil diameter by standardized pupillometry in groups of healthy subjects (total N= 40, 22-59 years), we found pupil-size to be larger in women than in men ($p<.01$), decreasing with age ($p<.05$) and varying with time of day ($p<.05$) and season ($p=.02$, nadir in winter). Also individuals that reported themselves as 'seasonal' had larger pupils as compared to 'non seasonals'.

Based on these findings, it is discussed to what extent pupil-size related processes can explain the known prevalence of winter depression in relation to gender (74-94 % are women) and age (winter depressives being generally younger than non seasonal depressives) as well as its seasonal link.

35

ADENYLATE CYCLASE INHIBITION BY PLASMA MEMBRANE OXIDOREDUCTASES IN SYNAPTOSOMES

O. Moulet & J.L. Dreyer, Institute of Biochemistry, University of Fribourg, CH-1700 Fribourg

The influence of beef brain synaptic plasma membrane oxidoreductases (OX) transcelular signal transduction has been investigated. The effects of OX on adenylate cyclase activity (AC) were tested under various experimental conditions. Activation of OX with nicotinamide pyridine nucleotides induces a small but reproducible decrease in the production of intracellular cAMP. AC is markedly affected by electron acceptors such as DCIP or quinones (Q). 1 mM DCIP induces over 70% inhibition of AC activity and cAMP production whereas Q under similar conditions inhibits AC up to ca 95% Various Q and electron acceptors (ferricyanide, glutathion, etc) were tested but no direct correlation between the redox potential of the electron acceptor and AC inhibition was found.

36

INFLUENCE OF EXTRACELLULAR MATRIX ON ENZYMATIC ACTIVITIES EXPRESSION OF CEREBRAL AND NON-CEREBRAL ENDOTHELIAL CELL LINES

Juillerat-Jeanneret,L., Janzer,R.C.,Division of Neuropathologie, University of Lausanne, 1011 Lausanne

Blood-brain-barrier function in the central nervous system is a property of endothelial cells, which can be induced and maintained in vivo by astrocytes surrounding the endothelial cells. In order to test the influence of extracellular matrix on specific markers associated with endothelial cells of cerebral origin we have measured specific enzymatic activities in endothelial cell lines of cerebral and non-cerebral origin grown on extracellular matrices secreted by cells of glial, fibroblastic and endothelial origin.

It was never possible to induce gamma-glutamyl-transpeptidase (gGTP) activity in cells of non cerebral origin. In a cerebral endothelial cell line constitutively expressing low levels of gGTP, extracellular matrices of 3 glioma cell lines decrease specifically gGTP and Leu-uptake. In another cerebral endothelial cell line with high basic expression of gGTP the same matrices have no effect on gGTP-expression and increase specifically Leu-uptake. All the other tested extracellular matrices had no major effects on gGTP-expression. In conclusion extracellular matrices of glial cell origin can modulate the expression of specific markers of brain endothelial cells depending on the state of differentiation of the endothelial cells.

37

SPIKE TRAIN ANALYSIS OF MONKEY INFEROTEMPORAL (IT) NEURONS DURING A DELAYED-MATCHING TASK. A.E.P. Villa and J.M. Fuster, *Dept. of Psychiatry and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024*

Recordings from IT cortex of monkeys performing a delayed-matching task showed that sustained firing elevations occur during the retention of a visual stimulus. A task-trial consisted of the following: (1) sample color; (2) 10-20 seconds of delay (retention period); and (3) choice of the color that matched the sample. Spike trains were selected from 134 single units activated during the delay. Time-series analyses were used to characterize the firing patterns. Out of 134 units, 29 fired tonically in the intertrial period; in the same period 105 exhibited bursts with intermingled and isolated spikes. The activation during the delay was selectively higher after one particular stimulus in 45/134 units (SEL units). The autocorrelogram was modified in 60% of SEL units vs. 24% of non-selective (NS) units. Repetitive patterns of firing, composed of 3 or more spikes, were found more frequently in NS than SEL units. In the NS group, the number of units with complex firing patterns was larger during the delay period (58%) than during spontaneous activity (39%). Conversely, SEL units exhibited fewer complex patterns during the retention period. This indicates that the temporal structure of the spike train as an important feature of IT neurons undergoes changes in visual short-term memory.

38

DECREASING PO_2 ENHANCES VISUAL INFORMATION PROCESSING

Schläpfer,Th. E., Fisch, H.-U., Dept. of Psychiatry, University of Berne, Murtenstrasse 21, 3010 Bern

High altitude (> 7000m) has a detrimental effect upon brain function; little, however, is known about the effects of acute hypoxia at lower altitudes despite the possibly greater importance.

Backward masking (BM), a reliable marker of visual information processing was used to assess cognitive performance at 550m and 3450m. A target letter (T) was presented tachistoscopically followed by a mask (M) composed of chopped letters. The temporal interval between the onset of T and M (Stimulus Onset Asynchrony = SOA) was the independent variable. Criterion was the percentage of correctly identified letters at each SOA. Athletic subjects (n=10, mean age 23.1 +/- SD 0.7 years) were trained at 550 m altitude (test-retest correlation for repeated BM curves r=0.97). After rapid (10 min) helicopter transport to the High Altitude Research Station on the Jungfraujoch (3450m), critical target duration for perception of unmasked T was reduced by 33 % and the number of correctly identified T increased ten-fold at SOA 20ms. ANOVA: SOA x Altitude ($F(5,45) = 7.9$, $p < 0.0001$), indicating an increase of performance above normal functioning.

One explanation for this paradoxic effect may be an increase in cerebral blood flow to compensate for the mild hypoxia. These findings indicate the impact of environmental variables on cognitive performance.

39

OPTICAL MAPPING OF ELECTRICALLY EVOKED ACTIVITY IN THE ANTENNAL LOBE OF THE HONEYBEE

D. Richard, M.G. Rioult and B. Palouzier-Paulignan; Laboratoire de Neurobiologie de l'Insecte, URA-CNRS 664, University Paul Sabatier, F-31062 Toulouse, France;
Dept. of Physiology, University of Bern, CH-3012 Bern, Switzerland.

Optical recording were used to study the organization of electrical activity in antennal lobe of honeybee (*apis mellifera*) evoked by stimulation of the antennal nerve.

Antennal lobes were isolated by microdissection and stained with di-4-ANEPPS, a fluorescent voltage-sensitive dye, at a concentration of 20 μM for 15 minutes. Electrical stimulation was performed with two 'en passant' suction electrodes on the antennal nerve. Changes in fluorescence intensity were recorded simultaneously by photodiodes (PD) of a 12x12 elements PD array mounted in the image plane of an inverted microscope. Each PD recorded activity from a surface of 24 μm x 24 μm. The signals were amplified and digitized at a maximal rate of 3.0 kHz/PD for up to 300 msec. Locally evoked activity was monitored along the fiber bundles of the antennal nerve up to the glomerulus of the antennal lobe. Although the presented results are preliminary ones, optical recording appear as a promising tool to study the spatio-temporal organization of information processing in the insect brain e.g. of olfactory afferences in the antennal lobe of the honeybee.

(Supported by CNRS and SNF: 'Projet sur convention internationale' 83F-028248)

40

QUANTAL RELEASE OF ACETYLCHOLINE BY SYNAPTOSOMES OF THE *TORPEDO* ELECTRIC ORGAN.

Girod R., Eder L., Medilanski J. and Dunant Y., Dpt. Pharmacol., C.M.U., 1211 Genève 4, **Tabti N. and Poo M-m.**, Dpt. Biol. Sci., Columbia University, N.Y. 10027, USA.

Transmitter release by isolated nerve terminals, or synaptosomes, has always been triggered and assayed biochemically. It is therefore not known whether in such extensively used preparation the fundamental property of intact nerve endings to release the transmitter in a quantal manner is retained. This quantal release is recorded in all intact synapses so far studied as fast spontaneous miniature potentials or currents generated in the postsynaptic cell. The purpose of the present work was to electrophsysiologically test whether purely cholinergic synaptosomes isolated from the *Torpedo* electric organ release acetylcholine (ACh) in such a discontinuous way. Synaptosomes were thus laid down on a culture of *Xenopus* embryonic muscle cells, which are equipped with nicotinic receptors and were used as detectors of the ACh released by the synaptosomes. Whole-cell recording in one of the myocytes revealed spontaneous synaptic currents (SSCs) that were generated soon after synaptosome application. The SSCs produced by the *Torpedo* synaptosomes were blocked by tubocurarine, indicating that they were due to pulsatile release of ACh. These SSCs resembled those occurring during the early phase of synaptogenesis in *Xenopus* cell cultures. It is concluded that synaptosomes retain *in vitro* their capacity to release ACh in a discontinuous, quantal manner.

41

MODELLING OF ACETYLCHOLINE ACTION AT THE ENPLATE

Correges, P.[1], Girod, R.[2], Jacquet, J.[2] and **Dunant, Y.[2]**, [1]Fac. Méd., Uni. Créteil, Paris, [2]Dpt. Pharmacol., C.M.U., 1211 Genève 4.

In the frog and mammalian neuromuscular junction, and in the nerve-electroplaque synapses of the *Torpedo* electric organ, the release of acetylcholine (ACh) has been demonstrated to be quantal. The quantum of transmission contains a few thousands of molecules of ACh and may be spontaneously released from the nerve endings. Its action on the postsynaptic cell is recorded as miniature end plate potential (Mepp) or current (Mepc). We developed a mathematical model that simulates diffusion of ACh in the synaptic cleft, its binding to the nicotinic receptors, its hydrolysis by ACh-esterase and its diffusion out of the cleft. Several kinetic and geometrical parameters were adjusted as to obtain a theoretical profile of nicotinic receptors opening and closing fitting reasonably well with the observed Mepps. The model reproduced the positive correlation between rise time and amplitude of Mepps, the effects of ACh-esterase inhibition and of partial blockade of the nicotinic receptor. When the initial concentration of ACh was increased, the model predicted a cooperative action of the transmitter on the postsynaptic membrane. By testing several temporal and spatial patterns of presynaptic ACh release, we simulated Mepps with variable time courses or notches on their rising phases, events which may be observed at cholinergic synapses. We are currently analysing different hypothesis concerning the molecular mechanism of ACh release.

42

Neurotransmitter release from Xenopus oocytes injected with mRNA from cholinergic neurons.

A. Cavalli, Y. Dunant, F. Loctin, (Dep. of Pharmacology CMU 1211 Geneve 4); N. Morel (CNRS, Gif-sur-Yvette);M. M. Poo, (Columbia Univ. New York).

The electric lobe of Torpedo fish contains the cholinergic cells bodies of the neurons that project to the electric organ. The oocytes injected with poly$^+$ (A) mRNA synthetize acetylcholine (Ach) from the precursor acetate. By stimulating oocytes with high potassium, we demonstated a calcium-dependent transmitter release. We have evidence that the oocytes injected with mRNA also expressed the mediatophore, a membrane protein which is able to translocate Ach. In the case that the mediatophore is a sort of Ca-gated channel protein, it is not known whether it works continously or discontinously. We are therefore attempting to detect the release of Ach via the membrane of an embryonic muscle cell in culture (myoball). The myoball is moved near to the depolarised oocyte membrane in order to explore the sites where Ach release takes place.

43

INTRACELLULAR RECORDINGS FROM TUBEROMAMMILLARY NEURONES IN VITRO.

A. Khateb, M. Serafin, and M. Mühlethaler, *Dept. de Physiologie, CMU, 1211 Genève 4, Switzerland*

By their widespread projections throughout the brain, histaminergic neurones from the tuberomammillary nucleus (TMN) are thought to play a critical role in the control of arousal. Recently, rat TMN neurones were recorded in vitro in hypothalamic slices or explants and shown to resemble other aminergic cells (Haas and Reiner, J. Physiol., 1988; Green et al., J. Physiol., 1990). In order to investigate these neurones beyond their intrinsic membrane properties, i.e. in order to understand the way they integrate synaptic inputs, we have investigated in a first step whether such cells could survive in an isolated and perfused whole brain (IWB) of guinea-pig in vitro. Indeed, we found that TMN neurones survive very well in this preparation, and that their main properties were similar to what has been already described: a regular tonic spontaneous firing, broad action potentials (more than 2 ms) and a strong transient A-like rectification. In addition, the presence of a massive spontaneous synaptic activity and the possibility to activate these neurones synaptically from distant places in the brain (such as the brainstem reticular formation or the optic nerve), indicate that this model could be useful for the study of TMN neurones, in particular with respect to their role in the sleep-waking cycle. (supported by a Swiss NSF grant no. 31-26495.89)

44

GLUTAMATE MEDIATES A SLOW SYNAPTIC RESPONSE IN HIPPOCAMPAL SLICE CULTURES

Serge Charpak and Beat H. Gähwiler
Brain Research Institute, University of Zürich, August Forel-Strasse 1, CH-8029 Zürich, Switzerland.

In hippocampal slice cultures, **exogenous** glutamate induces a slow excitation of CA3 pyramidal cells, resulting from the depression of potassium currents mediated through the activation of ACPD/metabotropic receptors (Charpak et al., Nature, 1990, 347:765-767). We report here, that in the presence of ionotropic receptor blockers, **endogenous** glutamate, synaptically-released during mossy fibre stimulation, produced a slow depolarizing postsynaptic potential which was associated with a decrease in membrane conductance. Furthermore, it depressed the slow after-hyperpolarization following a train of action potentials and reduced accommodation during the action potential train. Under voltage-clamp, mossy fibre stimulation induced a slow voltage-dependent inward current which resembled that produced by application of exogenous ACPD or quisqualate, and which was occluded by these metabotropic agonists. We therefore suggest that synaptically-released GLU can induce two types of postsynaptic responses : a fast excitation through activation of ionotropic receptors and a slower excitation associated with inhibition of K^+ conductances through activation of metabotropic receptors.

45

COMPARISON OF THE PRE- AND POST-SYNAPTIC ACTIONS OF BACLOFEN AT $GABA_B$ RECEPTORS IN HIPPOCAMPAL SLICE CULTURES.

Scott M. Thompson and Beat H. Gähwiler.
Brain Research Institute, University of Zürich, 8029 Zürich

Baclofen is a selective agonist for $GABA_B$ receptors. In the hippocampus, baclofen has been shown to activate a postsynaptic K^+ conductance and to block IPSPs by decreasing presynaptic release. We have used microelectrode recording from hippocampal slice cultures to examine the pharmacological properties of the receptors mediating these actions, their distribution, and their mechanism of action. Monosynaptic IPSPs, elicited in the presence of glutamate receptor antagonists, were potently inhibited by baclofen. The $GABA_B$ agonist 3-aminopropylphosphinic acid mimicked baclofen in the activation of the postsynaptic K^+ conductance and inhibition of synaptic inhibition. The $GABA_B$ antagonist CGP 35 348 blocked the late $GABA_B$-mediated IPSP, as well as the action of baclofen at pre- and post-synaptic receptors. Baclofen was unable to activate postsynaptic K^+ conductance or to block IPSPs in the presence of 1 mM Ba^{2+}. These data suggest that 1) baclofen blocks GABA release by acting at $GABA_B$ receptors on inhibitory interneurons, 2) baclofen acts at pharmacologically indistinguishable receptors at pre- and post-synaptic sites, 3) baclofen exerts its effects at both sites through activation of a barium-sensitive K^+ conductance.

46

FREQUENCY-DEPENDENT DEPRESSION OF SYNAPTIC EFFICACY IN EMBRYONIC RAT SPINAL CORD SLICE CULTURES

Streit, J., Physiologisches Institut, CH-3012 Bern

The modulation of synaptic efficacy by variations of the presynaptic stimulus frequency is believed to be of great importance for the formation of function-determined circuits during development. To reveal a possible role of this phenomenon in the formation of spinal stretch reflex circuits, the effects of stimulus frequency on the efficacy of synapses between dorsal root ganglion (DRG) cells or spinal interneurons and motoneurons were investigated in an embryonic DRG - spinal cord - muscle coculture. EPSPs and IPSPs were recorded in motoneurons during stimulation of DRGs or of various sites in the spinal cord (SC), using conventional intracellular microelectrodes. EPSPs and IPSPs were depressed at frequencies between 1 and 10 Hz by up to 100% (10Hz). Polysynaptic potentials decreased at lower frequencies than monosynaptic potentials. Composite EPSPs and single unit EPSPs from DRG or SC afferents were all depressed in the same frequency range. When single unit EPSPs were depressed, their amplitudes fell in the same quantal steps as were seen during amplitude fluctuations at constant stimulus frequencies. However, large steps composed of multiple quanta were consistently found during depression, suggesting that groups of quantal events were switched off together. Preceding or in parallel to the depression of EPSPs, an increase in latency by ca. 1ms was observed at high frequencies. In contrast to the EPSP amplitudes, the latencies of monosynaptic connections showed no fluctuations. The same frequency dependent alterations of latencies, as well as total conduction block at 10Hz, were found for action potentials elicited in the periphery and recorded in DRG or SC afferent cells. From these findings it is concluded, that the synaptic depression was of presynaptic origin, and that it was most likely due to conduction failures in the axonal arborizations of the afferents.

47

HOW NOISE MASKS FLUCTUATIONS OF SMALL EPSPs

Stricker,Chr.,Lüscher,H.-R., Physiologisches Institut, Universität Bern, Bühlplatz 5, CH-3012 Bern

Transmission at central synapses shows fluctuations in consecutive amplitudes of excitatory postsynaptic potentials (EPSPs). Fluctuations may be measured as the variance time course of the EPSP. The sensitivity of variance analysis is tested by computer simulations in relation to background noise. Linear addition of signal and noise is assumed. If the noise is considered as the error of the measurement (μ), then the error of the average and variance time course is $\mu/n^{1/2}$ and $\mu^{2}*(2/n)^{1/2}$, respectively. For signal to noise ratios <0.2 the average time course detects the amplitude correctly. However, the variance time course does not demonstrate the fluctuations even after 1024 EPSP measurements, because the noise variance is larger than the variance of the EPSP. This might lead to wrong conclusions about missing fluctuations. Variance analysis has a low sensitivity for small signals and, therefore, interpretations drawn from such analysis must be evaluated carefully.

48

PERIOD DOUBLING, PSEUDO-PERIODICITY AND POSSIBLE CHAOS IN ACTION POTENTIAL PROPAGATION.

Lüscher, H.-R. and Shiner, J.S., Department of Physiology, University of Bern, 3012 Bern

Action potential propagation in morphologically realistic terminal arborizations was simulated using SPICE, a general purpose circuit simulation program (Lüscher and Shiner, Biophys. J. 58: 1990). Action potentials were elicited at intervals ranging from 1.0 - 10.0 ms. At long intervals, the action potentials reached all the synaptic boutons without failures. At shorter intervals period doubling occurred. At successive branch points cycle lengths of up to period 10 were observed. In some cases a periodic stimulation pattern led to pseudoperiodic and chaos-like behavior at branch points along the axon arborization. The observed propagation patterns are critically dependent on geometrical parameters of the arborizations. Thus the enormous structural diversity of terminal arborizations may transform trains of action potentials into distinct temporal and spatial activity patterns at the presynaptic level. Supported by SNF 3.265-0.85 and the Swiss MS-Society.

49

Relation between EPSP and motor unit size during muscle activation

Studer,L., Ruegg,D.G., Gabriel,J.-P., Physiol. bzw. Math. Institut der Universität,CH-1700 Freiburg.

The size principle of Henneman describes the recruitment order of motor units (MU) in a muscle which is activated. Consequently the contraction force of the MUs is negatively correlated with the amplitude of the corresponding motoneuronal EPSPs, but the degree of correlation is undefined by the size principle. Recent experimental findings which suggested a linear relation between the muscle force and the input to the motoneuronal pool impose more stringent conditions on the relation between EPSP and MU force. In this investigation we try to quantify this relation. In a first step of modeling, we consider a MU to have a simplified 'one-area' motoneuron (MN). This MU develops its tetanic contraction force f according to the all-or-none principle. The muscle force F(f) is then given by the sum of the forces of all active MUs with forces equal or less f. To establish the transfer function of a MU, an electrical model of the ionic currents across the cell membrane is developed. We find that during small muscle contractions the size of the motoneuronal EPSP of a MU with contraction force f decreases in an approximatively exponential relation which is nearly inversely proportional to the muscle force F(f).

50

MONO- AND POLYCLONAL ANTIBODIES TO LOCALIZE SULPHONYL AMINO ACID TRANSMITTER CANDIDATES

Tschopp, Ph., Grandes, P., Morino, P., Stierli, B., Cuénod, M. and Streit, P., Brain Research Institute, Univ. of Zürich, CH-8029 Zürich

Mono- and polyclonal mouse antibodies were raised to glutaraldehyde-linked sulphonyl amino acid-albumin conjugates and evaluated for the immunohistochemical localization of homocysteate, cysteate or taurine. Antibody specificity was assessed using various amino acids and peptides conjugated to rat brain protein as test antigens in a system that mimicked immunocytochemical tissue processing and staining procedures. In cerebellum and hippocampal formation of perfusion-fixed rats, certain glial elements were the tissue components predominantly labeled with the various 'anti-homocysteate' antibodies; some antibodies stained mostly glial processes while others labeled cell bodies. 'Anti-cysteate' antibodies showed a strong and specific reactivity in our test system but yielded no staining pattern. 'Anti-taurine' antibodies labeled Purkinje cells and cells in the infragranular zone of the dentate gyrus. The antibodies developed will be useful reagents for more extensive immunohistochemical studies.

Supported by Swiss National Foundation grants 3.389. 86 and 31-27822.89

51

ELECTRICALLY-INDUCED RELEASE OF EXCITATORY AMINO ACIDS FROM ACUTE HIPPOCAMPAL SLICES.

J.M.Klancnik, M.Cuénod, B.H.Gähwiler and K.Q.Do
Brain Research Inst., Univ. of Zürich.

Excitatory amino acids (EAAs), neurotransmitter candidates, are released from hippocampus by high-K^+, in a Ca^{2+}-dependent manner. To identify release from specific pathways, the present study examined release of EAAs upon electrical stimulation (4 min, 50 Hz, 100 sec pulse width) of Schaffer collaterals. 1-min, 20µl fractions of superfusate were collected, via a 300µm dia. cannula placed c.50µm over the CA1 stratum radiatum of acute rat hippocampal slices. Electrophysiological responses were recorded to ascertain slice viability. Analysis was performed by reversed phase HPLC, with o-phthalaldehyde derivatization. Stimulation increased aspartate levels consistantly, but glutamate levels only occasionally. Cysteine sulfinic acid and homocysteic acid levels also increased; this is the first study showing release of these sulphur containing EAAs upon pathway stimulation. All of the above effects were blocked by 1 µM TTX.

52

EFFECT OF INTERFERON ON CA3 PYRAMIDAL CELLS IN RAT HIPPOCAMPAL SLICE CULTURES

Michael Müller and Beat H. Gähwiler, *Brain Research Institute, University of Zürich, August Forel-Str. 1, CH-8029 Zürich*

The therapeutic use of interferon (IFN) is often accompanied by neurological side effects such as fever and influenza-like symptoms, fatigue, somnolence, suggesting a direct influence on the CNS. We report here the effects of recombinant murine IFN-gamma and IFN-alpha/beta on CA3 hippocampal pyramidal cells in vitro, studied with standard electrophysiological techniques. Both IFN-alpha/beta and IFN-gamma had an excitatory effect, increasing the rate of spontaneous action potential discharges in CA3 pyramidal cells. This effect was not antagonized by naloxone. IFN-alpha/beta was 10-20 times more potent than IFN-gamma. IFNs appeared to produce this excitation via several mechanisms, in particular reduction of inhibitory synaptic transmission. This was most likely a presynaptic effect, since the sensitivity to iontophoretically applied GABA did not change after application of IFN. Interestingly, the rate of spontaneous synaptic activity was increased while the amplitude of the evoked IPSPs was diminished. The slow onset of these effects suggests an indirect mechanism of action. The fact that a cytokine known for its immunologic properties can affect neurons in the CNS suggests the presence of communication pathways between the immune system and the CNS.

53

BRADYKININ-AND ATP-INDUCED MEMBRANE CURRENTS IN NGF-DIFFERENTIATED PC12 CELLS

R. Neuhaus, B. Reber & H. Reuter. Dept. of Pharmacology, University of Berne, CH-3010 Berne

The effects of bradykinin and ATP were investigated with the whole-cell patch clamp method at a membrane potential of -50 mV. Bradykinin (500 nM) induced a transient K outward current, probably activated by Ca release from internal stores via IP_3. This current was partially blocked by replacing internal K with Cs ions and, with 60 nM internal free Ca, a slowly inactivating inward current appeared. Its amplitude depended on the extracellular Ca concentration: The average current density increased from 5 fA/µm² with 1.8 mM Ca to 13 fA/µm² with 10 mM Ca. Absence of extracellular Ca (5 mM EGTA) decreased the current density to 2 fA/µm². Thus Ca seems to be the main charge carrier. The mechanism by which the ion channel is coupled to the B_2 receptor remains unclear. However, the current was reduced to about 10% when no Ca was present in the internal solution (10 mM EGTA). PC12 cells also respond to extracellular ATP with a transient inward current, partially carried by Ca ions. However, in contrast to bradykinin the current is reduced when external Ca is increased. By simultaneous recording of membrane current and intracellular free Ca with fura-2 we found that both, bradykinin and ATP-induced currents are accompanied by a rise of the intracellular free Ca concentration.

54

VASOPRESSIN GENERATES A PERSISTENT SODIUM CURRENT IN A MAMMALIAN MOTONEURONE.

Raggenbass M., Goumaz M., Tribollet E. and Dreifuss J.J. Département de Physiologie, Centre Médical Universitaire, 1211 Genève 4.

The facial nucleus of the newborn rat is rich in [^{3}H]vasopressin binding sites and vasopressin can excite facial neurones by interacting with V_1-type receptors. To investigate the mode of action of this peptide we carried out single-electrode voltage-clamp recordings in coronal brainstem slices. In antidromically identified facial motoneurones vasopressin generated an inward current, whose magnitude was concentration-related; the lowest peptide concentration still effective was 10 nM. The vasopressin-induced current was resistant to TTX and persisted in a low-calcium high-magnesium medium. It was sustained, was inward at all potentials tested (-120 to -25 mV) and increased in magnitude following depolarization. It was not due to the blockade of a potassium current since it did not reverse at hyperpolarized potentials and was not modified by the potassium channel blockers TEA, 4-AP, barium, cesium, quinine, glibenclamide and apamin. Also, this current was not affected by changes in the transmembrane chloride gradient. By contrast, it could be reduced by partially substituting extracellular sodium with equimolar N-methyl-D-glucamine or Tris. Our results suggest that vasopressin increases the excitability of facial motoneurones by generating a persistend, voltage-gated, sodium-dependent transmembrane current.

55

DOPAMINE RELEASE AND BINDING STUDIES OF D_1 AND D_2-RECEPTORS IN BOVINE RETINA

Bugnon, O. and Schorderet, M., Département de Pharmacologie, CMU, CH-1211 Genève 4

The release and/or the synthesis of endogenous dopamine (DA) were studied by HPLC with electrochemical detection, in pieces of bovine retina *in vitro*, during 20 min of incubation at 37 °C, with constant oxygenation (95% O_2, 5% CO_2). DA and/or other catecholamines, as well as precursors (*e.g.* L-DOPA) or metabolites (*e.g.* DOPAC) were previously extracted by one-step purification on small Pasteur pipettes loaded with Sephadex G-10. The basal DA content was 2.87 ± 0,14 ng/mg protein (n=20), whereas noradrenaline was never detected. Potassium (K^+) (6.2-60 mM) and tyramine (0-100 µM) induced a dose-dependent release of DA *via* apparent different mechanisms. The effects of K^+ (50 mM) and tyramine (50 µM) appeared to be additive. The influence of calcium, DA-uptake inhibitors, D_2-agonists or -antagonists was investigated on K^+- and/or tyramine-induced DA release. In addition, binding studies of D_1- and D_2-receptors were carried out with specific ligands such as [H^3]-SCH 23390 and [H^3]-raclopride, respectively, in retinal membrane preparations. Both subtypes of DA receptors, possibly linked to the process of DA synthesis and/or release, were characterized by saturation and competition experiments.

56

I-123 ANALOG OF SCH-23390, A POTENTIAL DOPAMINE D_1 RECEPTOR MAPPING COMPOUND

Maier A., Schlumpf M., Lin S., Beer H.F., Schubiger P.A. and Lichtensteiger W., Inst. of Pharmacology, Univ. of Zürich, Zürich, and Paul Scherrer Inst., Radiopharmacy Div., Villigen PSI

Continuing the work with brain imaging tracers the iodo analog of SCH 23390 was labelled with I-123 for the use of SPECT analysis. The substance is known to bind specifically and with high affinity to the Dopamine D_1 receptors in rat brain. The method of labelling was an electrophilic substitution at C-7 of deschloro Sch 23390 R(+) enantiomer. With IODO-GEN and PBS (pH 7.4) the best labelling yield was obtained. The reaction mixture was purified by RP-18 HPLC. The product fraction was worked up to give, after sterile filtration, a ready for use radiopharmaceutical. The pharmakokinetics of the I-123 analog of SCH 23390 were tested in adult female Wistar rats after i.p. injection at the time of 10, 30, 60 and 180 min. The ratio of the whole brain/blood decreased from 1.23 to 0.5 during this time period. The main excretion path was through liver and intestine. The stomach showed an increasing uptake. Binding and displacement studies were carried out with adult male Long Evans rats. The binding to membranes of striatum exhibited the expected characteristic parameters of a substance that recognizes D_1-receptors.

57

GABA$_A$-BENZODIAZEPINE RECEPTOR STRUCTURE AND CHANNEL FUNCTION

Sigel, E., Baur, R., and Malherbe, P.$^+$, Pharmakologisches Institut der Universität Bern, CH-3010 Bern; $^+$Hoffmann-La Roche, Basel

Different combinations of cloned rat brain subunit isoforms of the $GABA_A$ receptor channel were functionally expressed in Xenopus oocytes. Electrophysiological techniques were then used to characterize the GABA-induced membrane currents and to study the effects of various modulators of the $GABA_A$ receptor channel (diazepam, DMCM, zolpidem, pentobarbital, picrotoxin, steroid metabolites). This approach was used to obtain information on the minimal structural requirements for several functional properties of the ion channel. The combination $\alpha5\beta2\gamma2$ was identified as the minimal requirement reproducing consensus properties of the vertebrate $GABA_A$ receptor channel, including cooperativity of GABA dependent channel gating with a K_a in the range of 10 µM, modulation by various drugs acting at the benzodiazepine binding site, picrotoxin sensitivity and barbiturate effects. The α-isoform present in a given subunit combination had a pronounced effect on several functional parameters. Therefore, we attempted to localize functional features within the α-subunit polypeptide by studying the functional properties of engineered chimeric α-subunits expressed in combination with β2 and γ2.

58

SIMILARITIES AND DIFFERENCES BETWEEN THE EFFECTS INDUCED BY $GABA_B$ RECEPTOR AGONISTS AND γ-HYDROXY-BUTYRIC ACID (GHB).

Rubio, V., Bernasconi, R., Leonhardt, T., Martin, P., Schmutz, M., Bittiger, H., CIBA-GEIGY Ltd., CH-4002 Basel.

To test the hypothesis that GHB exerts its effects through affinity for $GABA_B$ receptors we used CGP 35348, a $GABA_B$ receptor antagonist, to block the action of GHB on the rate of GABA synthesis and on cerebellar levels of cGMP. Similar to the $GABA_B$ receptor agonist baclofen, GHB decreased dose-dependently cGMP levels, but in contrast to baclofen, the effect of GHB was only partially antagonized by CGP 35348. Baclofen and GHB dose-dependently decreased the rate of GABA synthesis. However, CGP 35348 only blocked the decrease caused by baclofen and not that elicited by GHB.

These results suggest that the biochemical and behavioural effects of GHB result only in part from its interactions with $GABA_B$ receptors.

59

PRENATAL DEVELOPMENT OF ALPHA1, BETA2 AND GAMMA2 SUBUNITS OF THE $GABA_A$ RECEPTOR IN THE RAT

Parmar R., Schlumpf M., Lichtensteiger W., Barth M., Rhyner T. and Möhler H., Institute of Pharmacology, University of Zürich

Regional ontogeny of mRNAs encoding α1, ß2 and γ2 subunits of the $GABA_A$ receptor were studied by *in situ* hybridization on 10µm cryostat sections of rat fetuses. Specific probes (60mer oligodeoxynucleotides) were labeled with ^{35}S using terminal deoxynucleotidyltransferase and dATP, and signals were visualized by autoradiography. The 3 probes revealed signals present at all levels of fetal spinal cord, where they appear in a ventrodorsal density gradient, that is most distinct for the γ2 probe signal. The 3 subunit-mRNAs share an early clear presence in colliculi, mesencephalon and thalamus. Differences in the appearance of the signals are noted in neocortex, striatum and olfactory bulb. In fetal striatum ß2-mRNA and to some extent γ2-mRNA are distinctly expressed while the signal for α1-mRNA is vague. In neocortex, α1 message appears in a narrow band in the outermost cortical layer(s), while the γ2- and even more so the ß2-mRNAs extend over several layers. These results point to ontogenetic differences in the receptor structure.

60

PAROXYSMAL INHIBITORY POTENTIALS MEDIATED BY $GABA_B$ RECEPTORS IN DISINHIBITED HIPPOCAMPAL SLICE CULTURES. **Massimo Scanziani, Beat H. Gähwiler, Scott M. Thompson.** Brain Research Institute, Univ. of Zürich, 8029 Zürich

We have studied the involvement of $GABA_B$ receptors in the regulation of hippocampal excitability. The $GABA_B$ antagonist CGP 35 348 blocked the late IPSP in hippocampal slice cultures without causing epilepsy, unlike $GABA_A$ antagonists, such as bicuculline. Application of low concentrations of the glutamate antagonist CNQX changed bicuculline-induced epileptic activity in CA3 pyramidal cells into an cycle of epileptic bursts alternating with EPSPs followed by a long-lasting inhibitory potential. This paroxysmal inhibitory potential (PIP) was as large as 30mV, had a reversal potential near E_{K^+}, was prolonged by the GABA-uptake blocker nipecotic acid, and was reduced by the opioid-agonist FK 33-824. CGP 35 348 abolished the response, indicating that PIPs are mediated by the activation of $GABA_B$ receptors. In contrast to control $GABA_B$ IPSPs, the amplitude of the PIP was reduced by the NMDA-receptor antagonist D-APV. Low concentrations of bicuculline increased the amplitude of the control $GABA_B$-mediated synaptic potential 2-3 fold. These responses showed similar properties as the PIP. However, application of CGP 35 348 under these conditions lead to intense epileptic discharge.The PIP may therefore be a self-protecting mechanism preventing epileptogenesis after partial disinhibition.

61

RELATIONSHIP BETWEEN BENZODIAZEPINE RECEPTOR (BZR) OCCUPANCY AND EFFECT *IN VITRO* AND *IN VIVO* OF TRIAZOLAM, DIAZEPAM, Ro 19-8022 AND BRETAZENIL

Facklam M., Schoch P. and Haefely W., Pharmaceutical Research Department/CNS, F. Hoffmann-la Roche Ltd., CH-4002 Basel

We determined the relative intrinsic efficacy of four BZR ligands. *In vitro*, concentration-effect curves for the potentiation of 10 µM GABA-stimulated 36-chloride flux into membrane vesicles of rat cerebral cortex were measured and correlated with BZR occupancy. Whereas the curves are hyperbolic (superlinear) and virtually superimposable for diazepam and triazolam, Ro 19-8022 and bretazenil exhibited supralinear characteristics. *In vivo*, fractional BZR occupancy in mice was related to the anticonvulsant activity (prevention of audiogenic and PTZ-induced seizures) and to motor impairment assessed in the rotarod test. In these paradigms triazolam showed half-maximal effect at 5, 15 and 20% occupancy; diazepam at 15, 40 and 50%; Ro 19-8022 at 35, 90 and ~ 100%, while bretazenil needed 65 and ~ 100% for anticonvulsant activity and was inactive in the motor performance test. Thus, Ro 19-8022 and bretazenil were partial agonists *in vitro* and *in vivo*. Triazolam and diazepam behaved as full agonists *in vitro* but triazolam had a higher apparent intrinsic efficacy *in vivo*.

62

OSCILLATORY ACTIVITY INDUCED BY NMDA AND APAMIN IN THE MEDIAL VESTIBULAR NUCLEI.

M. Serafin, A. Khateb, C. de Waele*, P.P. Vidal*, and M. Mühlethaler, *Dept. de Physiologie, CMU, 1211 Genève 4, Switzerland and *Laboratoire de Physiologie Neurosensorielle, CNRS, Paris, France.*

We have recently identified in the guinea-pig medial vestibular nuclei (MVN) two main neuronal cell types, A and B MVNn, differing by their intrinsic membrane properties. One subtype of B MVNn was further characterized by the presence of low threshold calcium spikes (LTS). Both A and B MVNn were depolarized by NMDA, which also induced a decrease in membrane resistance and an increase in the spontaneous firing. These effects could be blocked by D-AP5, a specific antagonist of NMDA receptors. Following a 10-30 mV hyperpolarization, a long-lasting oscillatory bursting behavior could be induced in presence of NMDA. These oscillations were however restricted to the subtype of B MVNn without LTS. The NMDA-induced oscillations were TTX-resistant, but could be eliminated either by D-AP5, or by replacing sodium with choline, or by adding cobalt, a calcium channel blocker. Another similar type of oscillation could be triggered with apamin (10-7 M), presumably by blocking calcium-activated potassium conductances. However these oscillations were resistant to D-AP5 and could be eliminated by TTX. It is speculated that these oscillations might play a role in the rhythmic firing of vestibulospinal neurones during locomotion (Supported by a Swiss NSF grant no. 31-26495.89 and the French Ministère des Affaires Etrangères).

63

THE EFFECT OF SYSTEMIC NMDA RECEPTOR ANTAGONISTS ON A DELAYED TIME DISCRIMINATION TASK IN RATS

S. Berz, H. Welzl, K. Bättig, ETH Zürich, Lab. Behavioral Biology, CH-8092 Zürich

We investigated how the noncompetitive NMDA antagonist MK 801 and the competitive NMDA antagonist CGS 19755 affect performance efficiency in a delayed time discrimination task in the rat. Animals had to discriminate between two light signals of 2 sec. or 8 sec. duration. After a varying delay (0 to 8 sec.), they were rewarded with a food pellet for a correct lever press. Activity (nose pokes into the food tray), reaction time (time from opportunity to lever press to actual lever press), and performance efficiency (% correct responses) were measured. The results suggest that MK 801 and CGS 19755 differentially affect performance efficiency and activity in this task. Both drugs decreased efficiency at higher doses (0.2mg and 3.0mg/kg, respectively). However, whereas MK 801 had a slight stimulating effect (0.05 mg and 0.1 mg/ kg) on activity, CGS 19755 (1.0 mg and 3.0 mg/kg) decreased activity. Reaction time was only slightly increased by a higher dose of CGS 19755 (3.0mg/kg). Supported by NF grant No. 3.184-0.88.

64

COMPETITIVE NMDA (N-METHYL-D-ASPARTATE) RECEPTOR ANTAGONISTS BLOCK EXCITOTOXIC BRAIN DAMAGE IN VIVO

Massieu, L., Thedinga, K.H, McVey, M. & Fagg, G.E. Ciba-Geigy Ltd., CH-4002 Basel.

NMDA (N-methyl-D-aspartate) receptor mechanisms have been shown to be involved in the neurodegenerative changes following cerebral ischaemia. We evaluated the neuroprotective properties of competitive (CGP 37849) and uncompetitive (MK 801) NMDA antagonists vs quinolinic acid-induced damage in the rat striatum, using the enzymes, choline acetyltransferase and glutamate decarboxylase, to monitor neuronal damage. When co-injected into the striatum with quinolinic acid (200 nmol), CGP 37849 was a highly potent neuroprotectant, with an ED_{50} of 0.22 nmol; MK 801 was weaker, with an ED_{50} of 140 nmol. In contrast, when administered i.p., MK 801 was a more potent neuroprotectant than CGP 37849 (ED_{50}'s 0.65 and 17 mg/kg, respectively), probably reflecting differences in blood-brain barrier penetration of the two substances. Both blockers were able to prevent striatal damage when administered 1-2 h post-quinolinic acid injection. Drugs of this type may be of value for prevention of brain damage resulting from excessive glutamate release and NMDA receptor activation.

65

INVESTIGATION OF AF102b IN VARIOUS MODELS FOR M1 AND M3 MUSCARINIC RECEPTORS (MR).

A. Enz, M. Lichtsteiner* and H.W.G.M. Boddeke, SANDOZ Pharma AG, Preclinical Research, CH-4002 Basle, *Psychiatric University Clinic, CH-4025 Basle, Switzerland.

AF102b a putatively selective M1 agonist was tested in in vitro models for M1 (rat cervical ganglion;RCG) and M3 MR (guinea-pig ileum;GPI) and in A9 L cells transfected with cloned m1 or m3 MR. Radioligand binding with AF102b was performed using CHO-1 cells which express cloned m1 or m3 MR. AF102b induced concentration-dependent depolarizations in RCG (pD_2= 6.1; efficacy (eff.)= 79%; eff. of muscarine=100). AF102b acts in GPI as a partial agonist with respect to force of contraction (pD_2= 4.9, eff.= 52%). AF102b increased PI turnover (pD_2=4.5 (m1) and 4.9 (m3) respectively and intracellular [Ca] in the A9 L cells (pD_2=4.3 (m1) and 4.5 (m3); eff.=10 and 20% respectively). In CHO-1 cells AF102b displaced [^{3}H]NMS monophasically (pK_i: 5.5 (m1) and 5.4 (m3)). We conclude that AF102b is a selective M1 agonist in conventional in vitro systems. In cells transfected with cloned m1 or m3 MR, however, AF102b is a weak, non-selective partial agonist. At present we have no explanation for the different potencies in the respective M1 and M3 models and the lack of selectivity of AF102b at cloned MR.

66

AFFINITY AND DESENSITIZATION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS

Adrian Gross, Marc Ballivet$^+$ and Daniel Bertrand, Department of Physiology, CMU and $^+$Department of Biochemistry, Sciences II, 1211 Geneva 4

Two different neuronal nicotinic acetylcholine receptors (nAChRs) were expressed in *Xenopus* oocytes after nuclear injection of cDNA expression vectors. The subunits α4 and α3 were separately coexpressed with the structural subunit nα1. Both combinations yielded functional channels of neuronal type (Couturier et al, J. Biol. Chem., in press). These two receptor subtypes were readily distinguishable from one another by ACh sensitivity and desensitization. Receptors constituted with the α3 subunit showed lower acetylcholine sensitivity and stronger desensitization than receptors made with the α4 subunit. Since the subunit nα1 was unchanged, we conclude that the α subunits must be responsible for these functional differences. Hybrid cDNAs were constructed that contained the N-terminal sequence down to the first transmembrane segment of α4 and the remaining sequence of α3, and vice versa. These hybrids, named α4:α3 and α3:α4, produced functional receptors when co-injected with nα1. Data obtained with these hybrid receptors indicate that ACh sensitivity is mainly defined by the first extracellular sequence of the α subunit. We are presently investigating whether the observed differences in desensitization between α4 and α3 depend upon their intracellular sequences.

67

FUNCTIONAL PROPERTIES OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS CONTAINING DIFFERENT β SUBUNITS

Cachelin, A.B.[1], Sigel, E.[1] and Jaggi, R.[2]. [1] Pharmakologisches Institut der Universität Bern, 3010 Bern [2] Abteilung f. klinisch-experimentelle Forschung der Universität Bern, 3004 Bern.

We have used the two-electrode voltage-clamp method to investigate the effect of various agonists on neuronal nicotinic acetycholine receptors expressed in *Xenopus* oocytes following injection of either α3-β2 or α3-β4 cRNAs. Fast application of micromolar concentrations of either acetylcholine (ACh) or 1,1,Dimethyl-4-piperazine (DMPP) yielded currents of several μA amplitude. The activation of either receptor types by DMPP showed cooperativity (Hill-coefficient n = 1.7) and was half-maximal at concentrations between 10-20 μM. In α3-β4 receptors, ACh also displayed cooperativity (n=1.7) but was somewhat less potent than DMPP. Desensitization was much more pronounced in α3-β2 than in α3-β4 receptors, causing waning of a significant proportion the DMPP-induced response within the first seconds of agonist application.

Supported by a Roche Research Foundation grant to A.B.C.

68

STEROIDS INHIBIT NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS

Valera, S., Bertrand, S., Ballivet, M., Rungger, D., Bertrand, D., Biochemistry, Physiology (CMU) and Animal Biology Dpts., University of Geneva, 1211 Genève 4, Switzerland.

There is good evidence that steroids modulate ligand-gated channels such as $GABA_A$ and glycine receptors. We therefore examined whether avian neuronal nicotinic acetylcholine receptors (nAChRs) can be modulated by steroids. Receptors were reconstituted into *Xenopus* oocytes following nuclear injection of cDNA expression vectors. Progesterone applied onto oocytes expressing α4/nα1 nAChR rapidly and reversibly reduced the current evoked by acetylcholine (ACh). At 50 nM ACh, the dose-response curve of this inhibition is described by a Hill equation (apparent K_i = 7 μM, and n = 0.6). Among the steroids tested progesterone was the most powerful inhibitor, while dexamethasone (a synthetic glucocorticoid) and 3α-OH-dihydroprogesterone (the most potent activator of $GABA_A$ receptors) were almost inactive. Preliminary results obtained with α7, a brain α-bungarotoxin sensitive receptor, suggest that channels formed with this subunit are also responsive to progesterone. These results demonstrate that nAChRs can be modulated by progesterone and suggest that steroids play an important role in nicotinic cholinoception.

69

α7, an alpha-bungarotoxin sensitive brain receptor.

D. Bertrand, S. Couturier, J.M. Matter, S. Bertrand, S. Wonnacott and M. Ballivet

Dpt of Physiology CMU, Dpt of Biochemistry Sciences II Geneva Switzerland
and Dpt of Biochemistry, Bath BA2 7AY UK.

The puzzling differences between the high-affinity bindings of alpha-bungarotoxin (α-Bgt) and nicotine to brain slices were partially elucidated by the isolation of a new gene encoding an α-Bgt binding protein subunit (α7) in chicken brain. Functional homo-oligomeric acetylcholine receptors were successfully reconstituted in Xenopus oocytes following intranuclear injection with α7 cDNA alone (Couturier et al., Neuron in press). From the correlation of the ACh-evoked current with the number of α-Bgt binding sites, we inferred that up to 10^7 receptors can be expressed by a single oocyte. The physiological and pharmacological properties of α7 significantly differ from those of all the other members of the neuronal nicotinic family: they are more sensitive to nicotine than to ACh and the neuronal nicotinic agonist 1,1-dimethyl-4-phenylpiperazinium iodide fails to evoke any response. These results demonstrate that the brain α-Bgt sensitive protein constitutes neuronal ACh receptors, whose function remains to be elucidated.

70

METABOLIC STABILIZATION OF SYNAPTIC AChR.s IS MEDIATED BY Ca^{++} INFLUX ASSOCIATED WITH MUSCLE ACTIVITY

S.Rotzler and H.R.Brenner, Physiologisches Institut der Universität, CH 4051 Basel.

During the formation of the neuromuscular junction, the synaptic AChR.s become metabolically stabilized, their half-lives increasing from about 1 day to about 10 days. This process is regulated by the electrical activity induced in the muscle by innervation. We have examined the mechanisms by which electrical activity induces AChR stabilization. Chronically denervated adult rat soleus muscles were treated in organ culture, pulse-labelled with ^{125}I-α-Butx and kept in culture for up to 3 days. AChR half-lives ($t_{1/2}$) were estimated from the decay of endplate-bound radioactivity with time. 24hrs of electrical stimulation in vitro was sufficient to stabilize the AChR.s from $t_{1/2}$=2.5d to $t_{1/2}$=13.1d. AChR-stabilization could also be induced in the absence of stimulation by treatment of muscles with the Calcium ionophore A23187 ($t_{1/2}$=14.4d) or with the Ca^{++} channel activator (+)-SDZ 202-791 in elevated K^+ ($t_{1/2}$=7.6d). Conversely, the stabilization of AChR's was prevented in muscles stimulated in the presence of the Ca^{++} channel blockers (+)-PN 200-110 or D 600. Treatment of the muscles with ryanodine to induce release of Ca^{++} from the sarcoplasmic reticulum did not cause stabilization of junctional AChR's. These data show that the activity-dependent AChR stabilization is mediated by an influx of Ca^{++} ions through dihydropyridine-sensitive Ca^{++} channels in the muscle membrane.

71

HISTAMINERGIC RECEPTORS IN THE MEDIAL VESTIBULAR NUCLEUS OF THE GUINEA-PIG. M. Mühlethaler, A. Khateb, N. Vibert*, C. de Waele*, P.P. Vidal*, and M. Serafin, *Dept. de Physiologie, CMU, 1211 Genève 4, Switzerland and *Laboratoire de Physiologie Neurosensorielle, CNRS, Paris, France.*

We have recently shown that NMDA receptors contribute to the resting discharge of vestibular nuclei neurones (de Waele et al., Exp. Brain Res., 1990). In the same perspective, we have now investigated in vivo and in vitro the effects of histamine, a transmitter originating from the tuberomammillary nuclei and known to play a role during arousal.

In vitro, we have shown earlier that two main neuronal cell types are present in the medial vestibular nucleus, corresponding to the tonic and phasic neurones described in vivo. Histamine (through a direct action) induced in both cell types a small membrane depolarization with a minor decrease in membrane resistance but a large increase in firing. In both cell types, these effects were mimicked by Impromidine (a specific H2 agonist) and blocked by Cimetidine (an H2 antagonist). Both H1 and H3 analogues (antagonists and agonists) had no effects.

In vivo, local perfusion of the vestibular nuclei with H3 analogues induced a strong ipsilateral postural syndrome. Altogether, we propose that histamine could exert a control over the vestibular system by acting on postsynaptic H2 receptors, the spontaneous release of histamine in vivo being under the control of inhibitory H3 autoreceptors. This might explain the effects of antihistaminergic drugs acting in motion sickness (Swiss NSF 31-26495.89, French Ministère des Affaires Etrangères).

72

VAGUS NERVE SECTION INDUCES IPSILATERAL DISAPPEARANCE OF OXYTOCIN BINDING SITES IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS NERVE OF THE RAT.

Dubois-Dauphin M., Tribollet E., and Dreifuss J.J. Département de Physiologie, Centre Médical Universitaire, 1211 Genève 4

The dorsal motor nucleus of the vagus nerve (DMN) of the rat receives oxytocin (OT) immunoreactive axones derived from cell bodies located in the hypothalamic paraventricular nucleus. Vagal motoneurones send their axon to visceral ganglia through the ipsilateral vagus nerve. Applications of OT into the DMN causes neuronal excitation and affects gastrointestinal functions. OT binding sites are detected in DMN, whereas the neighbouring nucleus of the solitary tract (NST) is endowed with vasopressin (AVP) binding sites. To assess the pre- or postsynaptic location of the binding sites in DMN and NST, we cut unilaterally the vagus nerve distally to the nodose ganglion. Two weeks later, the presence of binding sites was assessed in cryostat sections by film autoradiography using ^{3}H-AVP and ^{125}I-OTA as ligands. In lesioned animals, as in controls, the same density of AVP binding was present bilaterally in the NST. In contrast, OT binding sites were fewer or absent altogether from the DMN ipsilateral to the cut vagus nerve. A strong axon reaction was seen in the ipsilateral DMN, which was revealed by cresyl violet staining and choline acetyltransferase immunocytochemistry. These data suggest that OT synthetized in the hypothalamus acts directly in DMN upon the vagal motoneurones.

73

A COMPARISON OF BINDING SITES FOR VASOPRESSIN LOCATED ON CORTICOTROPHS IN RAT, SHEEP AND HUMAN PITUITARIES.

Du Pasquier D., Dreifuss J.J., Loup F., Dubois-Dauphin, M. and Tribollet, E. Département de Physiologie, Centre Médical Universitaire, 1211 Genève 4.

Vasopressin (AVP) carried by hypophysial portal blood acts at the pituitary level, in synergy with CRF, to induce ACTH secretion. The aim of the present work was to map the binding sites for AVP in rat, sheep and human pituitaries, and to establish whether they are associated with corticotrophs. To this end, we obtained contact autoradiographs from 15 µm thick cryostat sections using 1.5 nM [^{3}H]AVP as the ligand. ACTH-immunoreactivity was detected on sections immediately adjacent to those used for autoradiography. In all three species, specific binding sites for AVP were detected in the anterior lobe - the intermediate lobe was not labelled and the neural lobe non-specifically. Experiments using the radioligand in competition with synthetic structural analogues showed that pituitary binding sites differ in structural requirements from both the V_1 and V_2 receptor subtypes. Specific binding sites formed an irregular, patchy pattern throughout the anterior lobe. In all three species, this pattern was strikingly similar to that formed by cell clusters showing ACTH-immunoreactivity. [^{3}H]AVP binding was more intense in the sheep and human anterior lobe than in the rat, suggesting that AVP may play a more dominant role in controlling ACTH secretion in the former than in the latter species.

74

THE USE OF ANTI-IDIOTYPIC ANTIBODIES FOR IMMUNOHISTOCHEMICAL PROOF OF ANGIOTENSIN II RECEPTORS IN RAT BRAIN

Pfister,J., Felix D. and Imboden H., Universität Bern, Abteilung Neurobiologie, Erlachstrasse 9a, 3012 Bern

The renin-angiotensin system in the brain plays a decisive role in the functioning of the hypothalamus, angiotensin II (ANG II) probably being the biologically active substance within this system. Using the peroxidase-anti-peroxidase method and an anti-idiotypic antibody against ANG II-receptors, we examined immunohistochemically the distribution of these receptors in the supraoptic nucleus (SON) and the paraventricular nucleus (PVN). For purposes of comparison adjacent brain slices were examined with a specific antibody against ANG II for the presence of this peptide. In the areas of the SON examined 90% of the neurones showed ANG II-receptors and 57% ANG II. In the magnocellular areas of the PVN there was an abundance of neurones which had ANG II-receptors, while only a few contained ANG II. An examination of individual cells from the SON and the PVN showed 1) neurones with ANG II-receptors but no ANG II, and 2) neurones with ANG II-receptors and ANG II.
Supported by SNF grant 31-29986.90.

75

NEUROPEPTIDE Y (NPY) POTENTIATES ANGIOTENSIN II (AII)-INDUCED VASOCONSTRICTION AND ACCUMULATION OF INOSITOL PHOSPHATES (IP).

F.Cressier and K.G. Hofbauer, CVS Dept, Ciba-Geigy Ltd., Basel, CH.

NPY has a direct vasoconstrictor action and potentiates the effects of other vasopressor hormones. To elucidate the type of NPY receptor and the effector mechanisms involved we studied the influence of NPY (acting on Y1-and Y2-receptors) and NPY 13-36 (acting only on Y2-receptors) on the effects of AII. We used the rabbit femoral artery to measure vasoconstriction and accumulation of IP. The vascular effects of AII (1 nM) were potentiated by NPY (0.05 µM).NPY 13-36 had no effect. NPY (1 µM) potentiated the AII-induced dose-dependent(0.01-1.0 µM) formation of IP, without having any effect by itself. In contrast, NPY 13-36 did not affect the AII response. In conclusion, NPY potentiates the AII-induced vasoconstriction and accumulation of IP. This effect is probably mediated via Y1-receptors. Whether the rise in IP is due to receptor activation or an increase in intracellular Ca2+ needs to be evaluated.

76

INHIBITION OF THE GLUCOCORTICOID RECEPTOR FUNCTION BY THE ONCOGENE PRODUCTS OF *fos* AND *jun*

R. Jaggi[1], M. Touray[1], F. Ryan[2], B. Jehn[1], A. Marti[1] and F. Martin[2] [1] Universität Bern, Abt. für klin.-exp. Forschung, Tiefenaustr. 120, 3004 Bern. [2] Pharmacology Dept., University College Dublin, Ireland

In NIH 3T3 cells overexpression of Fos or Fos/Jun (AP-1) inhibits glucocorticoid-dependent gene expression. The inhibition can be overcome by overexpression of the glucocorticoid receptor (GR). In receptor deficient CV-1 cells glucocorticoid-dependent reporter gene expression is induced by a range of functional GR truncation mutants. C/D domain of the receptor which encompasses the DNA-binding, a transactivational and a dimerisation domain is a sufficient target for inhibition by Fos and Fos/Jun. When present simultaneously in the cell nucleus the glucocorticoid receptor and Fos and Jun form a specific and stable protein/protein complex. The GR interacts physically with both, Fos and Jun, when cotranslated *in vitro*. Our findings suggest cross-talk between two distinct transcription factors in the cell nucleus resulting in the inhibition of glucocorticoid receptor function. We are studying the inhibitory effect by Fos/Jun on other members of the steroid hormone receptor family. Individual members of the Jun family of oncogenes have the potential to form heterodimers by associating with Fos. We are investigating the role of individual members of Fos and Jun in the inhibition of glucocorticoid receptor function.

77

THE RAT SAPHENOUS NERVE NEUROMA - A MODEL TO INVESTIGATE MECHANISMS IN NERVE GROWTH FACTOR (NGF) AND NGF RECEPTOR REGULATION

Ehrhard, P. and Otten, U., Dept. of Physiology, University of Basel, CH-4051 Basel

The most proximal segment of the transected saphenous nerve, a stump neuroma, was used to characterize mechanisms in NGF and NGF receptor synthesis. In saphenous nerve neuromata of adult rats a long-term increase in NGF protein was detected by a NGF ELISA after axotomy. There was a rapid 4-fold increase in NGF levels 6 hr after injury, which reached peak values after 4 days and subsequently fell to 2-fold elevated levels within 3 weeks. Quantitative Northern blots showed that NGF mRNA levels increased rapidly, reaching a maximum (30-fold) 12 hr after transection, indicating that the increase in NGF in response to injury is due to local biosynthesis. Moreover, by using a sensitive immunoprecipitation assay for NGF receptors we found that levels of receptor start to increase 7 days after lesion and remained elevated for up to 7 weeks after nerve transection. These data indicate that after nerve transection NGF and NGF receptors are induced at sites proximal to the axotomy.

78

DETECTION OF G PROTEIN-COUPLED RECEPTOR SEQUENCES BY PCR AMPLIFICATION USING A SINGLE DEGENERATED AMPLIMER

Marie-Claire Combe, Miguel Munoz and Philippe Walker
Division of Hypertension, CHUV, CH-1011 Lausanne

Adrenergic receptors, acetylcholine muscarinic receptors, tachykinin receptors, dopamine receptors, the thyrotropin receptor and few others send their signal to the interior of the cell through a transducing G protein. All these G protein-coupled receptors share three common structural features: 1) seven sequences (TM1 to TM7) of 22 to 24 hydrophobic amino acids thought to form transmembrane α helixes; 2) a large hydrophilic loop between α helixes 5 and 6; and 3) a hydrophilic C-terminus. In addition, these receptors contain DNA sequence homologies within some of the TM domains. *Using PCR amplification, we have generated a probe for the detection of G* protein-coupled receptors expressed in a neuroblastoma cell line (SK-N-MC). PCR amplification of total SK-N-MC library cDNA was carried out with a degenerated amplimer homologous to TM6 and a fixed amplimer corresponding to a vector sequence flanking the 5' end of inserts. The labelled PCR products used as probes light up sequences coding for α2 and β2 adrenergic receptors, the substance K receptor and a muscarinic receptor but neither the MAS oncogene nor negative controls such as actin sequences or vector fragments. This technique is applicable to other cell types and could be used to identify a broad spectrum of G protein-coupled receptor sequences.

79

Cellular proteins binding to the prion protein

Oesch, B. and Prusiner, S.B., Brain Research Institute, Univ. of Zürich, August-Forel-Str. 1, 8029 Zürich and University of California, Dept. of Neurol., San Francisco, CA 94143

The scrapie-specific isoform of the prion protein (PrP^{Sc}) is part of the infectious particles causing scrapie (in sheep), Creutzfeldt-Jakob-Disease (in humans), and bovine spongiform encephalopathy (in cattle). In order to analyze the mechanism of transmission and/or replication of the infectious agent (denominated prion) we have characterized cellular proteins which interact with the cellular or scrapie prion protein on ligand blots. Two PrP-binding proteins of 45 and 110 kDa (denominated Pli 45 and Pli 110) were present in scrapie-infected and normal hamster brain. Pli 45 was more abundant in scrapie than in normal brain. Biochemical purification and sequencing of Pli 45 revealed its identity with glial fibrillary acidic protein (GFAP) which is increased in scrapie-infected brain. The intracellular localization of PrP^{Sc}, as opposed to the cell surface localization of cellular PrP, may therefore be a consequence of the interaction of PrP^{Sc} with the cytoplasmic intermediate filament protein GFAP. Future experiments will show whether astrocytes which produce GFAP play an important role in prion diseases.

80

Regional and developmental analysis of amyloid precursor proteins (APPs) in rat brain.

J. Löffler, A. Schuler and G. Huber. Pharma Research Dept., F.Hoffmann-La Roche Ltd, CH-4002 Basel

The ß/A4-protein deposited in senile plaques of Alzheimer's Disease is derived from a member of a family of precursor proteins(APP), some of which lacking and some containing a Kunitz type protease inhibitor domain. We are studying the expression of APP in rat brain in order to elucidate the distribution of different molecular forms in various brain regions as well as during brain development. With an antibody against the protease inhibitor domain a 120 kDa protein is found to be enriched in adult olfactory bulb. During development from newborn to aged rat (3 years old), no obvious changes in the total amount of this form are detectable.

An antibody against the N-terminal 16 aminoacids of the ß/A4-protein, recognizing membrane-bound as well as normally secreted forms of APP with and without protease inhibitor domain, detects multiple proteins of around 110 kDa and one protein of 60 kDa. These proteins are equally present in all brain regions examined. An additional 85 kDa band is detected only in hippocampus, cerebral neocortex, and striatum. Most of these ß/A4 immunoreactive soluble forms decrease during brain development.

81

REMYELINATION STUDIES IN AGGREGATING BRAIN CELL CULTURES.

V. Comte, P. Honegger and J.-M. Matthieu.
Laboratoire de Neurochimie, Service de Pédiatrie,
Centre Hospitalier Universitaire Vaudois, 1011 Lausanne.

Demyelination in aggregating brain cell cultures can be induced, in the presence of complement, with a monoclonal antibody (8-18C5) directed against myelin/oligodendrocyte glycoprotein (MOG), an external surface protein. After demyelination, aggregates were washed and returned to normal media conditions. Remyelination was observed by measuring myelin basic protein (MBP), 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP) and glutamine synthetase (GS), an astrocyte enzyme, as control. After 2 days in demyelinating conditions, MBP concentration decreased to 35% of control. The MBP level reached 50% of control after 2 days of recovery and 93% of control after 7 days. CNP specific activity decreased too, but less drastically and continued to decrease 7 days after having removed anti-MOG antibody. GS specific activity was not affected indicating a specific effect on oligodendrocytes. Bovine growth hormone (bGH), platelet-derived growth factor (PDGF) and triiodothyronine (T_3) have been shown to promote myelination, both *in vivo* and *in vitro*. In the present study, bGH and T_3 did not improve remyelination. In contrast, low concentrations of PDGF seemed to have a positive effect on remyelination, whereas high concentrations seemed to be inhibitory. The absence of response to bGH and/or T_3 suggests that these factors stimulate differentiation of immature oligodendrocytes and have no effect on mature, differentiated oligodendrocytes which, in this study, were responsible for remyelination.

82

ONTOGENY OF SOMATOSTATIN GENE EXPRESSION IN RAT BRAIN.

J.-M. BURGUNDER, Neurologische Klinik, University of Berne, CH-3010 Berne, Switzerland.

Somatostatin (SRIF) has been localized in several neuronal populations of the adult mammalian brain and functions during ontogeny have been postulated. In the present study, the development of SRIF gene expression was examined at the cellular level in the rat brain using hybridization histochemistry. SRIF mRNA was detected in several neuronal populations of the basal diencephalon (anterior and posterior) and basal telencephalon (lateral) for the first time on E14. On E16 a prominent increase of the extent and level of expression was found in these areas. In addition cells in the medial telencephalon and a few cells in the future allocortex also contained SRIF mRNA for the first time. In the prenatal period, the expression in the above cells continued to mature and individual nuclei began to be recognizable. At birth the overall pattern of SRIF gene expression was established but the ventral portions (hypothalamus, amygdala, allocortical areas) had much higher levels of expression than the more dorsal ones (striatum and neocortex). Over the first two weeks after birth, this difference diminished and an adult-like pattern was found at postnatal day 21.