The effect of stimulation of la-ryngeal and tracheal mechanore-ceptors and arterial baroreceptors on the airway resistancein humans

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The purpose of the present study was to investigate the influence of selective carotid baroreceptors activation and combined activation of baroreceptors and mechanoreceptors located in the larynx and upper part of trachea on the airway resistance in humans. Selective activation of baroreceptors was induced by generation of negative pressure (- 40 mm Hg) for 5 s. in the two capsules placed bilaterally on the neck over the carotid arteries bifurcation. The single chamber encompassing frontal and lateral parts of a neck was used for simultaneous activation of the baroreceptors and the upper airways mechanoreceptors. The above methods were validated by measurement of intra-oesophagal pressure. Negative pressure in the single chamber was transmitted in 50 % to the oesophagus, whereas the pressure changes in the single capsules were not reflected at oesophagal level. Selective stimulation of carotid baroreceptors caused decrease in airway resistance, whereas opposite was true when both baroreceptors and mechanoreceptors in upper airways were stimulated concomitantly. As atropine abolished the above changes in the airway resistance, it is suggested that cholinergic efferent pathway is responsible for their occurrence. We conclude that in humans reflexes originated from baroreceptors and mechanoreceptors in the larynx and trachea influence the airway resistance. In case of simultaneous activation of baroreceptors and mechanoreceptors of the upper airways the later one prevails the former.

Dysfunction of autonomic cardio-respiratory regulation in sleep apnoea syndrome

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The autonomic nervous system controls both cardio- and respiratory functions in humans. The reflex mechanisms, involved in cardiovascular and ventilatory control, are reflexes from arterial baro- and chemoreceptors. The reflex ventilatory response to progressive hypoxia and hypercapnia, the heart response to baroreceptor activation and inactivation were studied in obstructive sleep apnoea patients and compared with healthy volunteers. The ventilatory response to progressive isocapnic hypoxia was reduced in OSAS, whereas the ventilatory response to hypercapnia was not different from the reactions observed in healthy controls. A decrease of upper airway resistance during progressive hypoxia was also reduced in sleep apnoea patients. A significant decrease of baroreceptor reactivity was found in all OSAS patients. After 4 weeks of cPAP treatment the impaired reflex responses were partially reversible.
We conclude that the reduction of baro- and chemoreceptor responsiveness in OSAS seems to result from the typical OSAS symptoms and might be partially reversed with the betterment of sleep quality.

Symposium 4 - Neuroimaging

See pages 72 and 73

Symposium 5 - Neuroactive amino acids in CNS disorders

NEUROACTIVE AMINO ACIDS IN HEPATIC ENCEPHALOPATHY

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Both acute and chronic liver disease result in alterations of neuroactive brain amino acids. Studies in autopsied brain tissue from patients with chronic liver disease who died in hepatic coma reveal decreased concentrations of glutamate and aspartate. Studies in experimental animal models of hepatic encephalopathy (HE) using *in vivo* microdialysis provide evidence for increased extracellular concentrations of aspartate, taurine and aromatic amino acids. It has been suggested that alterations of CNS taurine could play a role in the pathogenesis of brain edema in acute liver failure. Quantitative receptor autoradiography reveals a generalized loss of the NMDA subclass of glutamate receptors both in experimental HE and in hyperammonemia. It has been suggested that HE in chronic liver failure results from alterations in neuron-astrocytic metabolic "trafficking" of neuroactive amino acids and their metabolites. Alterations of the GABA system in HE are restricted to changes in GABA-B receptor function. Despite early reports to the contrary, there is no currently substantial evidence to support the hypothesis that postsynaptic GABAergic function *per se* is altered in HE. On the other hand, a subgroup of patients with HE respond to treatment with flumazenil, a GABA-related benzodiazepine receptor antagonist suggesting that this system may be implicated in the pathogenesis of HE. (Funded by MRC Canada).

TAURINE IN THE HEALTHY AND DISEASED BRAIN

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Taurine, 2-aminoethanesulfonic acid, is a ubiquitous free amino acid in animal organisms, particularly abounding in the central nervous system during the early phases of ontogenesis. It has been demonstrated by us and others to modulate electrical activity and participate in the volume regulation of neural cells. Depolarizing stimuli evoke taurine release from neurons and hypoosmotic conditions both from neurons and glial cells. The evoked release is fairly massive in preparations from immature neural tissue. In hepatic encephalopathy the potassium-evoked release of taurine is diminished from the striatum. Excitatory amino acids induce a marked release of taurine in the brain. On the other hand, taurine has been shown to protect cultured neurons against excitotoxicity. It also assists neurons and astrocytes to adjust their cell volumes to different osmotic conditions. In epileptic foci the concentration of taurine generally tends to diminish, even though the data have been relatively inconsistent. Taurine also alleviates symptoms when administered as an drug in both animal seizure models and epileptic patients. It has been generally effective, however, only in about one third of patients in clinical trials. Taurine is a very lipophobic compound, poorly penetrates brain tissue and any excess of taurine in plasma is readily excreted into urine. We have therefore tested a series of novel taurine derivatives as putative antiepileptics. Several of them proved effective in animal seizure models, one of them, taltrimide (2-phthalimidoethanesulfon-N-isopropylamide, MY-117) has already been tested in a phase I clinical trials but failed to have any major beneficial effects as an auxiliary drug in intractable cases of epilepsy. In addition to epilepsy, taurine and taurine derivatives should, however, to be systematically investigated, e.g., as prophylactic agents in migraine and as protective drugs in hypoxic brain damage.

EXCITATORY AMINO ACIDS IN NEURODEGENERATIVE DISORDERS: GLUTAMATE AND ENERGY METABOLISM

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It is well established that glutamate receptors play a major role in mediating acute neuronal degeneration in the CNS in that cerebral ischemia and head or spinal cord trauma are associated with excessive release and extracellular accumulation of glutamate, which leads to persistent activation of glutamate receptors and acute neurotoxic degeneration of the hyperstimulated neuron. It has been more difficult to link neuronal degeneration that occurs in chronic neurodegenerative disorders to an excitotoxic mechanism. However, accumulating evidence suggests that impairment of intracellular energy metabolism may be a mechanism contributing to neuronal death in such disorders, and it is thought that hyperactivation of glutamate receptors may be involved. It is proposed that impaired energy metabolism results in deterioration of membrane function and loss of the voltage-dependent Mg^{2+} block of N-methyl-D-aspartate receptors, which allows persistent activation of these receptors by glutamate, even if concentrations of glutamate at the receptor are within the normal physiological range. Studies in rodents using mitochondrial respiratory chain toxins, such as aminooxyacetic acid, 1-methyl-4-phenylpyridinium ion, malonic acid and 3-nitropropionic acid, suggest that these agents do induce CNS degeneration by a process involving the excitotoxic mechanism. Striatal and nigral degeneration induced by mitochondrial toxins in rodents resembles neuropathology seen in humans suffering from Huntington's or Parkinson's disease, and can be attenuated by glutamate receptor antagonists and agents that improve energy metabolism. Such experimental observations suggest that disturbed energy metabolism and glutamate may be involved in neuronal death leading to abiotrophic/chronic neurodegenerative disorders in humans. If so, glutamate antagonists or agents to improve energy metabolism may slow the degenerative process and offer a therapeutic approach for temporarily retarding the progression of these disabling disorders.

EXCITATORY AMINO ACIDS, ANTIEPILEPTIC DRUGS, AND SEIZURE ACTIVITY

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Endogenous excitatory amino acids (e.g. glutamate or aspartate), as well as their synthetic analogues [e.g. N-methyl-D-aspartate (NMDA), quisqualate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)] are potent convulsants. Conversely, NMDA or AMPA receptor antagonists provide protection against various convulsive procedures. However, especially NMDA receptor antagonists may produce serious adverse effects when administered at anticonvulsive doses, so their potential use as antiepileptic drugs seems discouraging. Consequently, we attempted to study an influence of both NMDA and AMPA receptor antagonists on the anticonvulsive activity of conventional antiepileptics. The assumption was that excitatory amino acid antagonists might potentiate the protection offered by the antiepileptics and such an effect could be observed at the subprotective doses of these antagonists. This approach could eventually lead to the reduction of their adverse effects, the considerable anticonvulsive effect of the combined treatment still being retained. Indeed, when NMDA antagonists, CGP 37849 and CGP 39551 were combined with standard antiepileptics, a strong potentiation of the protective activity of these antiepileptics was noted against maximal electroshock-induced seizures in mice. However, other agents with NMDA receptor blocking properties (D-CPP-ene, memantine, procyclidine, trihexyphenidyl), although potentiated the protective action of conventional antiepileptics on one hand, produced distinct impairment of motor coordination and/or long-term memory on the other. Among AMPA/kainate receptor antagonists, both NBQX and GYKI 52466 enhanced the protective efficacy of conventional antiepileptics and the undesired effects of these combinations, in terms of motor and long-term memory impairment, were either absent or moderate. It is remarkable that in no case the potentiating effect of excitatory amino acid antagonists was associated with increases in plasma levels of antiepileptic drugs, indicating that a pharmacokinetic interaction is not probable. In conclusion, some excitatory amino acid antagonists may offer an advantage in the treatment of epilepsy when combined with conventional antiepileptics.

Excitatory amino acids and cysteine in development of brain damage afer neonatal hypoxia-ischemia.

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The aim of this study was to investigate the possible role of excitatory amino acids (EAA) and cysteine (Cys) in the development of brain damage after hypoxia-ischemia (HI) in neonates.

Rat pups were subjected to unilateral common carotid artery ligation and exposed to 1h or 1h 40min of hypoxia (7.7% O2). In dialysis experiments a 1mm dialysis probe was implanted into the parietal cortex of the HI hemisphere. Dialysates were collected from 1h before untill 6h after HI and analysed for amino acids (HPLC). Extracellular amino acids changes were correlated with the area of probe placement: (infarcted, undamaged and border zone evaluated 6h after insult). A pronounced extracellular overflow of glutamate (Glu) (15x), aspartate (Asp) (6x) and Cys (3x) was observed during HI and remained elevated during reperfusion (3x, 2x and 2x resp). During HI there were no differences in the pattern of EAA changes between the infarcted, undamaged and border zone regions. During reperfusion, however,the ec concentrations of Glu, Asp and Cys were higher in infarcted and border zone areas as compared to undamaged tissue. HI produced also a slight increase of tissue concentration of cysteine but not glutamate) in parietal cortex of the HI hemisphere at 1min and 4h reperfusion.

The effect of Glu and Cys on cell damage was evaluated in the arcuate nucleus. Glu (0.3, 0.5 and 0.8mg/g sc) induced dose-dependent neuronal necrosis after 4h. Cys alone (0.5mg/g sc) was not toxic, while its co-injection with Glu increased the damage. In on-going studies the direct effect of cysteine injection on HI-induced brain damage in neonates is investigated.

The results show that cysteine potantiates glutamate toxicity in neonatal rats. Speculatively, HI-induced release of Cys may enhance glutamate toxicity during reperfusion.

GLUTAMATE - A MEDIATOR OF INORGANIC MERCURY NEUROTOXICITY.

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In this report we describe evidence pointing to the indirect, glutamate (GLU)-mediated mechanism of neurotoxicity of mercuric chloride (MC). MC at 1 μM concentration (or less) inhibits astrocytic GLU uptake (Brookes, J. Neurochem., 50, 1117, 1988) and stimulates GLU release from astrocytes in culture (Mullaney et al., Dev. Brain Res., 75, 261, 1993). In vivo, these two events are thought to lead to accumulation of extracellular GLU at toxic concentrations. Inhibition of GLU uptake and stimulation of GLU release by MC are attenuated by a membrane-penetrating SH agent ditiothreitol (DTT) but not by glutathione (GSH) which is not transported to the inside of the cells, indicating that the MC-vulnerable groups are located in astrocytic membranes (Albrecht et al., Brain Res., 607, 249, 1993). Ultrastructural evidence for GLU-mediated MC neurotoxicity came from studies in an organotypic culture of rat cerebellum. We have shown that 1 μM MC lowers the threshold of GLU neurotoxicity (Matyja and Albrecht, Neurosci. Lett., 159, 155, 1993), and that the combined neurotoxic effect of GLU plus MC is attenuated by DTT but not by GSH (Matyja and Albrecht, Toxicol. In Vitro, in press), which is consistent with the involvement of astrocytic GLU transport. Moreover, neuronal damage induced in the culture by GLU plus MC becomes less accentuated in a medium with a noncompetitive NMDA receptor antagonist dizocilpine (MK-801).

Symposium 6 - Cellular and molecular events of CNS myelinogenesis

Cellular and Biochemical Events of CNS Myelinogenesis

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In the lecture following main problems, as the introduction to the session, will be discussed:

- The increased cell density during the myelination period should be regarded as a net effect of both the preliferative activity of the white matter glial cells and of the active migration of cells from the matrix.
- The increase of the enzyme acitvity in the neuroglie during myelination gliosis seems to be causatively connected to the metabolic processes of myelinogenesis.
- There exist a chronological order genetically determined in the synthesis of proteins and lipids during the onset of myelination.
- The genetic information is not the sole factor safeguarding the transformation of immature and mature oligodendroglia cells into myelinating cells.
- Trophic substances conveyed by means of axonal flow are essential factors controlling myelinogenesis by immature differentiating oligodendroglia cells.
- Oligodendroglia exhibit signal transduction systems that can be activated by classical neurotransmmitters.

DM-20 AS A MARKER OF THE OLIGODENDROCYTE LINEAGE

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Proteolipid protein (PLP) and its isoform DM-20, are major myelin proteins. While PLP is expressed only in mature oligodendrocytes, several observations suggest that DM-20 might be expressed earlier and thus serve as a marker of the oligodendroglial lineage during embryonic development. To test this hypothesis, we have undertaken the analysis of *DM-20* pattern of expression during embryonic development in two vertebrate species: the mouse and the chicken.

In the CNS of the mouse embryo, *DM-20* was first detected at E9.5 in the latero-basal plate of the diencephalon. At E12.5, this inital territory extended rostrally to the ventral hypothalamus. Caudally, *DM-20*$^+$ cells appeared in the latero-ventral plate of the rhombencephalon. At E14.5, the hypothalamic signal remained unchanged and in the diencephalon *DM-20*$^+$ cells were seen in the basal mantle layer. The number of *DM-20*$^+$ cells in the rhombencephalon had increased and extended all along the spinal cord. From E9.5 till E14.5, no *DM-20* signal was detected in the mesencephalon. Between E14.5 to birth, the number of *DM-20*$^+$ cells increased and progressively colonized the future major white matter tracts.

In the chicken embryo, the overall pattern of expression of *DM-20* was similar to the mouse. The first *DM-20*$^+$ cells were seen at E2 in the baso-lateral plate in the mesodiencephalic sulcus and the rhombencephalon, but no *DM-20* cells were seen in the mesencephalic curvature. At E3, the *DM-20* transcript was detected in the ventral hypothalamus. From E5, the *DM-20*$^+$ cells were seen in the ventral mantle layer, then there number progressively increased and they localized mostly in the future white matter tracts.

Double labeling experiments failed to show any colocalisation of *DM-20* with neuronal markers. In addition, culture and grafting experiments showed that *DM-20*$^+$ neuroepithelial territories generated oligodendrocytes. Altogether, these data suggest that *DM-20* is an early marker of the glial lineage and that embryonic *DM-20*$^+$ cells have a restricted potentiality to differentiate into oligodendrocytes.

ABSTRACT FORM

MECHANISMS OF MAG GENE ACTIVATION IN DIFFERENTIATING OLIGODENDROCYTE LINEAGE CELLS

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Myelin associated glycoprotein (MAG) plays an essential role in the formation and stabilization of periaxonal architecture of the myelin sheath. The upregulation of the MAG gene is contemporaneous with phenotypic differentiation of progenitor (O-2A) cells into myelin-producing oligodendrocytes.

The MAG gene chromatin from approximately -1.6 to +0.7 kb features MNase hypersensitivity that may delineate the gene control region. The core promoter is contained within approximately 150 bp upstream from the most 3' transcription start site. Because neither canonical TATA box, nor initiator motifs are present within this sequence, the MAG promoter represents a novel type of TATA-less promoters. Further upstream region contains potent enhancers that may be crucial for the oligodendrocyte-specific expression. The DNA around this region appears to be organized into an array of positioned nucleosomes with hypersensitive linkers. The core promoter itself is contained within a nucleosomal core particle with only the most 3'end protruding into the linker region. This core particle is displaced in the process of the gene activation. The MAG gene activation is also concomitant with profound demethylation of two CpG dinucleotides at -39 and -1,835 in postmitotic oligodendrocytes. Furthermore, the differentiating oligodendrocyte lineage cells undergo dramatic changes in nuclear *trans*-acting factors that bind to their cognate *cis*-elements within the core promoter region. Hence, the upregulation of the MAG gene entails both chromatin remodeling, and alteration in the assortment of unique *trans*-factors.

PT-RABBIT MUTATION: GENETIC CHARACTERISTIC AND PHENOTYPIC EXPRESSION.

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Genetic characteristic of pt-rabbit has been recently accomplished. This x-linked mutation influencing plp gene can be assumed as one of the known animal models of human Pelizaeus-Merzbacher disease. Rabbit plp cDNA has been sequenced, revealing transversion T - A in exon 2 of pt-mutation-affected gene. This point mutation, placed at DNA region encoding the first potential transmembrane domain of PLP (or DM20) protein results in a substitution of histamine^{36} by glutamine. The affected animals, although strictly controlled for pt trait, differ significantly in their phenotypes. The developmental expression of myelin specific proteins and glycolipid antigens in phenotypically different mutants has been investigated. The degree of CNS hypomyelination and underexpression of myelin specific markers correlated well with the severity of neurological symptoms and was highest in the most affected animals. This correlation was especially demonstrative in the case of PLP and DM20 protein expression. In addition to hypoexpression, there was a profund retardation in the developmental appearance of the most of investigated myelin-oligodendrocyte specific markers.
It is concluded that PLP abnormality in pt rabbits affects not only and even not preferentially the myelin structure but disturbs the normal oligodendrocytes maturation. It means that from initially equal number of unmatured glia cells only a minute fraction in pt brain could pass the more advanced stages to the phenotypes expressing all components needed to proper myelin synthesis and assambly. The mechanism(s) responsible for the ability of certain population of oligodendrocytes (different in pt individuals) to overcame this mutation-guided block of differentiation is not known at present.

Do Oligodendroglia Divide?

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It is firmly established that remyelination occurs in the adult central nervous system following a wide variety of experimental and naturally occurring demyelinating conditions. These include immune mediated diseases such as multiple sclerosis and experimental autoimmune encephalomyelitis, and demyelination induced by toxins, such as ethidium bromide and lysolecithin. Remyelination is preceded by the generation of new oligodendrocytes. The source of these new oligodendrocytes is now known. They could be generated from glial precursor cells known to exist in adult brain; or from pre-existing differentiated oligodendrocytes that re-enter the cell cycle, possibly first dedifferentiating; or both processes may occur. Much evidence for and against these possibilities derives from both *in vivo* and *in vitro* studies. This evidence will be presented and discussed. In the light of our own studies that show that proliferating astrocytes in injured adult brain arise from pre-existing astrocytes, not from glial precursor cells.

γδ T CELLS AND HEAT SHOCK PROTEINS IN MULTIPLE SCLEROSIS

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We studied immunoreactivity for heat shock proteins (Hsps) in multiple sclerosis (MS) brain tissue and detected hsp-65 in chronic MS plaques at the edge of thinly myelinated areas. Double staining revealed that hsp-65$^+$ cells represented reactive, immature oligodendrocytes. Mature oligodendrocytes outside MS plaques did not stain for hsp-65. Control brain sections howed no hsp-65 reactivity. We defected immunoreactivity for hsp-72 in astrocytes in MS and non-MS brain to a similar extent but no association between hsp-72 reactivity and MS plaques was evident. Oligodendrocytes expressed also hsp-65 in mixed glial cultures. In MS tissue, hsp-65 $^+$ oligodendrocytes colocalized with T lymphocytes expressing the γδ T cell receptor (TCR). The coexpression of these molecules might imply functional relationship perhaps of significance to the chronicity of the MS disease process. Some TCRγδ cells had an unusual morphology with long dendritic processes. Using PCR analysis, γδ T cell presence in MS lesions was confirmed. The δ chain gene rearrangement patterns expressed were Vδ2-Jδ3 and Vδ2-Jδ1. Using direct sequencing, we found a striking predominant gene rearrangement within the Vδ2-Jδ3 TCR population that was detected in all of the MS patients and was not present in CNS tissue from other neurological diseases. The sequence of the predominant Vδ2-Jδ3 gene rearrangement was confirmed by cloning and sequencing. This junctional region was characterized by shortened Dδ and Jδ segments, few N region insertions, and predominant usage of the third reading frame of the Dδ3 segment. Since this region (CDR3 region) of the TCR is thought to be primarilly involved in antigen recognition, these data support the conclusion that γδ T cells within the MS lesion are responding to a common antigen.

Plenary lectures

THE ATTENTIONAL MODE OF THE VISUAL SYSTEM

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The stayed hypothesis that descending feedback projections in the visual system might be activated during attentional processes was recently supported by observation that cortico-geniculate synapses have a built-in frequency amplification mechanism. We have found that when the cortical discharge rate approaches 20 Hz a mechanism is activated that depolarizes the lateral geniculate nucleus (LGN) principal cells and therefore increases the input-output gain of the geniculate relay (Lindström and Wróbel 1990). Since layer 6 pyramidal cells, remain silent during anaesthesia, as was shown by Livingstone and Hubel (1981), it is therefore worthwhile investigating the role of the descending pathways in awake animals. Subsequently, we succeeded to show that cats required to attend during a visual discrimination task showed an enhanced neuronal activation within cortical and geniculate centers which consisted of short bursts (300 - 1000 ms) of 20 Hz oscillations. With the use of Directed Transfer Functions we were able to show that this activity was propagated from the visual cortex toward the LGN as predicted by the feedback hypothesis, and also towards other parts of the primary visual cortex (Bekisz and Wróbel 1993, Wróbel et al. 1994). Furthermore the oscillatory rhythm which seems to be moderately synchronized all over the visual system during nonvisual situations changes towards a highly specific pattern of synchronization during attentive seeing. We suggest that such a pattern of 20 Hz beta activity represents a temporary activated mosaic of functional connections needed by the current visual scan.

The proposed hypothesis for the role of the feedback pathways in attentive perception is appealing because it can also be applied to higher stages of visual processing as well as to other sensory systems.

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Genetic regulation of CNS glutamate receptors

P.H. Seeburg, Heidelberg

Not received

Workshop 4 - Cortical processing

Cortical Systems for Processing Auditory, Somatosensory, and Visual Information in Primates

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Comparative studies of the organization of sensory cortex in prosimian galagos, several species of New World monkeys, and Old World monkeys reveal basic components of the auditory, somatosensory, and visual systems that appear to exist in all primates, including humans, and variable components present in some monkeys. In simians, auditory cortex can be characterized as having a core of primary-like areas, a belt of secondary areas, and a parabelt of higher areas. The core includes A1, R, and RT, fields with medial geniculate inputs and outputs to a surrounding array of 7 or more fields in the belt, some of which project to the parabelt which relays to frontal cortex. Somatosensory cortex in all primates includes a primary area, S1 or area 3b, an adjoining area for deep receptors, 3a, a caudal fringe area that is elaborated into areas 1 and 2 in simians, and at least two lateral areas, S2 and PV. All primates have visual areas V1, V2, MT, DM and DL, and possibly other fields. Prosimians appear to have less visual cortex, but differences in fields remain uncertain.

Location and action of amino acid neurotransmitter receptors at identified synapses in the cerebral cortex. Peter Somogyi MRC, Anatomical Neuropharmacology Unit, Mansfield Road, University of Oxford, OX1 3TH, U.K.

About 17% of boutons release GABA and the rest of the synapses release glutamate as transmitter. Synaptic inputs from different sources, but using the same transmitter are segregated on the surface of cortical cells as best seen in the hippocampal formation, where excitatory and inhibitory afferents terminate in conjunction on specific dendritic segments. Two sets of experiments will be reported:

i/ paired intracellular recordings in vitro from synaptically coupled cortical cells together with the visualisation of synapses. Both *axo-axonic* and *basket cells* act through $GABA_A$ receptors but they innervate, respectively, the axon initial segment or the soma/proximal dendritic region of principal cells. Other cells activating $GABA_A$ receptors have most of their output restricted to dendrites in conjunction with excitatory inputs. Postsynaptically, unitary IPSPs evoked by most identified neurons are mediated exclusively by $GABA_A$ receptors, whereas presynaptic use-dependent depression of release is governed by $GABA_B$ receptors (1). Unitary responses fluctuate, but show infrequent release failures, due to the relatively large number (6-15) of electron microscopically determined synaptic release sites (1). These principles apply to both hippocampal and neocortical circuits. In the CA1 area basket cells can produce a unitary IPSP followed by rebound excitation in pyramidal cells which are close to firing threshold. This rebound was sufficient to produce action potentials in the postsynaptic cells. Fast unitary IPSPs elicited by a single anatomically identified basket cell could rapidly phase lock postsynaptic pyramidal cells. We propose that basket cells may act to synchronise the activity of large populations of principal cells.

ii/*high resolution immunogold localisation of receptors.* All immunocytochemical methods reveal an abundance of receptors for GABA and glutamate in the extrasynaptic membrane of neurons and glia, but only the postembedding immunogold method is suitable to reveal receptors in synaptic junction. All subunits of the AMPA type glutamate receptors are concentrated in the postsynaptic membrane specialisation in the hippocampus, and are present at synapses receiving glutamate from distinct sources. Postsynaptic ionotropic and metabotropic glutamate receptors (mGluRs) are segregated (2). Immunolabelling for mGluRs is concentrated in a perisynaptic annulus at the edge of synaptic junctions. Extrasynaptic mGluRs are concentrated in dendritic spines. We propose that irregularly shaped, "perforated" synapses develop in order to increase the metabotropic/ionotropic glutamate receptor ratio. The perisynaptic location of mGluRs may result in their activation only at repeated release of glutamate. $GABA_A$ receptors are concentrated mainly in type II postsynaptic membrane specialisations (3). The synaptic/extrasynaptic immunolabelling density ratio for $GABA_A$ receptors is several hundred fold. In hippocampal principal cells some of the same $GABA_A$ receptor subunits are used at synapses receiving GABA from distinct presynaptic sources (3). In conclusion, cell types differ in the density and variety of receptors, but on the same neuron, the same receptor mechanism and subunits are used in different synaptic junctions. The density and location of receptors is highly compartmentalised. (1) Buhl, E.H. et al. 1994. *Nature* **368**, 823-828. (2) Nusser, Z. et al. 1994. *Neuroscience* **61**, 421-427. (3) Nusser, Z. et al. 1994. *Eur J Neurosci* 7.

MAPPING OF STIMULUS FEATURES AND MEANING IN GERBIL AUDITORY CORTEX

H. SCHEICH, H. STARK, W. ZUSCHRATTER, F. OHL AND C. SIMONIS, Federal Institute for Neurobiology (IfN), Magdeburg, 39118, Germany

Among at least seven fields in gerbil auditory cortex the primary field (Al) and the anterior auditory field (AAF) showed prominent tonotopic organization with parallel dorsoventral iso-frequency contours. Aversive tone conditioning paradigms reshaped frequency receptive fields of single units and also changed the spatial representation of tones in fluoro-2-deoxyglucose experiments (FDG). This suggests that spectral features as well as aspects of behavioural meaning of sounds may be represented in Al and AAF through learning.

As one aspect of plasticity antibodies against the immediate early gene product c-Fos identify the spatial distribution of neurons in auditory cortex which presumably change metabolism as a result of stimulation with novel auditory signals. Less than 3 min training with a novel tone led to tonotopic columnar expression of c-Fos in Al. Longer stimulation led to spreading of c-Fos expression across auditory cortex. The search for transmitters which mediate this gene activation is greatly aided by microdialysis through chronically implanted probes in auditory cortex. So far, metabolites of dopamine and serotonine transmission were found to reflect specific aspects of auditory avoidance conditioning in a shuttle box. Notably, dopamine release appeared to parallel the speep improvement of performance during the first training session and distinguished learning animals from unpaired controls.

Coherence and bicoherence analysis of subdural and intracerebral EEG for assessment of cortical dynamics

J. Achimowicz, Th. Bullock, Warsaw

Not received

Workshop 5 - Psychophysiology of cognitive functions

Psychophysiology of Cognitive Processes: Central and Autonomic Measures

Robert J. Barry, Department of Psychology, University of Wollongong, Wollongong, Australia

A brief overview of the history of the Orienting Reflex (OR) in Western Psychology is presented, in order to provide a context for a discussion of its role in attentional processing. Some aspects of observed response fractionation are discussed, leading to an outline of a coherent theory of preliminary processes in OR elicitation. This discriminates between involuntary and voluntary aspects of cognitive processing but depends on a common core mechanism. The role of state variables in modulating phasic responses is also discussed. Although this theory was developed largely from a study of automatic responses, it has been possible to extend it to include various central measures, and recent extensions are described. A number of recent studies are briefly outlined to provide examples demonstrating the use of a range of physiological measures (central and peripheral) in a variety of situations (from the pistol range to the laboratory) with different subject groups (athletes, children and psychiatric patients). These examples indicate the wide-ranging potential benefits of using psychophysiological approaches in the study of cognitive processes. Finally, the use of heart rate data in the investigation of task-relevant cognitive load is discussed as a relatively simple but sensitive index to explore drug and other effects.

Evoked Cardiac Response (ECR) as a Function of Interaction Between Processing Load and Reaction Time (RT) Individual Differences.

Kaiser, J. (1), Beauvale, A. (2), Bener, J (1).

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ABSTRACT
The paper reports research on the phasic HR changes in the relation to RELEVANT and IRRELEVANT stimuli in subjects with short and long RT. Both groups received 10 innocuous auditory stimuli with randomly varying interstimulus intervals. Stimuli were presented in one of two conditions defined by the instruction to count the stimuli (RELEVANT) or lack of the instruction (IRRELEVANT). Mean values of HR were obtained at .5 s intervals for 30s epochs commencing 20s before each stimulus. HR was corrected for respiratory sinus arrhythmia.
In IRRELEVANT condition a mean HR response has shown a decelerative characteristics. In the case of long RT subjects a deceleration has been significantly deeper if compared with short RT subjects. In RELEVANT condition in comparison to IRRELEVANT condition an interactive effect as the additional acceleration has been obtained. Long RT subjects have presented bigger above effect when compared to the opposite group.
These findings are explained in terms of individual differences in the domain of cognitive processing intensity related to capacity of task performance.

HEART RATE CHANGES DURING COGNITIVE ACTIVITY IN SUBJECTS WITH DIFFERENT LEVEL OF INTELLIGENCE

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The aim of the experiment was to analyze wheather HR is the physiological index, sensitive to changes in mental workload in Ss with different level of cognitive abilities. By means of Raven's Matrices Test, a sample of 90 participants was selected from large college students population - 45 Ss was identified as highly intelligent, whereas the remaining 45 Ss had an average level of intelligence. Ss were randomly assigned to the experimental conditions with different mental workload. Its magnitude was varied using two manipulations: the cognitive demands imposed by a task were low or high (easy vs. difficult task) and emotional load of the situation (time pressure during difficult task was present or absent). All Ss performed in counterbalanced order two tasks: so-called perceptual (trials from Cattell's „Culture Fair Intelligence Test") and arithmetic via symbols (in arithmetic expressions numbers should by substituted by geometrical symbols). It appeared, as expected, that despite the mental workload magnitude as well as the type of cognitive task, Ss with high intelligence were more efficient in task performance, especially when perceptual task was taken into the consideration. At the same time, on the psychological level (state anxiety assessed by Spielberger's STAI), those individuals were affected by time pressure much more than Ss with average level of intelligence. As for physiological reactions, baseline tonic HR level in two groups showed no significant difference. With regard to phasic heart rate reactions (5-sec. periods following immediately response were derived from continuous HR recording) it appeared that despite the mental workload, individuals with high level of intelligence consequently showed significant, greater cardiovascular changes during cognitive activity than Ss with average level of intelligence. That difference between two groups of Ss was especially pronounced for arithmetic task. The data obtained suggest that during mental activity emerge differences on psychological as well as physiological level between persons differing in psychometrically measured intelligence.

Hemispheric Asymmetry for Visual Information Processing

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The left and right hemispheres of humans do not handle all aspects of visual information processing with equal ability. Instead, the two hemispheres operate in a complementary fashion, with each being better able than its partner to handle certain aspects of visual information. This is illustrated by a review of research concerning the processing of (1) global versus local stimulus properties, (2) low versus high spatial frequencies and (3) coordinate versus categorical spatial relationships. In general, the right hemisphere is dominant for processing global aspects of visual stimuli that are carried by low spatial frequencies and for processing coordinate spatial relationships. The left hemisphere is dominant for processing local aspects of visual stimuli that are carried by high spatial frequencies and, perhaps, for processing categorical spatial relationships. Consideration is given to developmental mechanisms that may underlie the emergence of hemispheric asymmetry for these interrelated aspects of visual information processing.

This presentation is included in the session entitled "Psychophysiology of Cognitive Functions."

Joseph Hellige is an Invited Speaker

DIRECTIONAL DIFFERENCES IN INTERHEMISPHERIC TRANSMISSION TIME

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The study investigated whether the interhemispheric transmission of information depends on hemispheric specialization. Visual evoked potentials (VEPs) were recorded in response to words and gratings exposed for 30 ms, in random order, in the left and right visual fields. The subjects' task was to judge whether the laterally presented stimulus was present on a response card (exposed 500 ms after the stimulus onset) or not. Electrodes were located over the left and right occipital lobes at O1 and O2 according to the 10/20 system and referenced to linked ear lobes. Two VEPs components, N170 and P300, were analyzed. N170 had larger amplitudes and shorter latencies when recorded from the directly stimulated hemisphere (i.e., contralateral to the field of stimulus presentation) than when recorded from the hemisphere stimulated via corpus callosum (i.e., ipsilateral to the field of stimulus presentation). Analysis of P300 did not show significant effect of either the field of stimulus presentation or the stimulated hemisphere. The latency differences in N170 registered in the ipsilateral and contralateral hemispheres were the basis for comparison of interhemispheric transmission time (IHTT) in the two directions: from the left hemisphere to the right and from the right hemisphere to the left. In the case of words, shorter IHTTs were observed when the information was transferred from the right hemisphere to the left, whereas in the case of gratings IHTTs were shorter when the information was transferred from the left hemisphere to the right. The results showed, thus, that interhemispheric transmission time was shorter when the information was transferred *from* the hemisphere non-specialized for its processing *to* the specialized one than in the opposite direction. The results suggest the existence of a physiological mechanism that ensures fast transmission of information to that hemisphere which is more efficient in its processing.

Temporal constraints in processing of nonspeech rhythmic patterns.

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Temporal integration is one of the mechanisms at the highest level of a hierarchical taxonomy of time perception which allows to integrate information over time and to bind successive temporally separated events into one unit. On the basis of neuropsychological evidence it can be concluded that the temporal extent of this mechanism is limited to approximately 2-3 sec. The present study investigated the effect of a mental content of presented stimuli, normal aging and individual differences in cognitive abilities on temporal limits of an integration mechanism. Younger and older subjects integrated the temporal information contained in continuous metronome beats. The subjects were asked to listen to the rhythm of metronome and to accentuate mentally every second, third, fourth ... etc. beat in order to hear a subjective rhythm. This rhythm existed, in fact, only in subjects' mind and not objectively. The subjects reported verbally how many clicks they could integrate into a perceptual unit. On this basis, the time interval in which the subjects were able to integrate the information was calculated (number of beats reported as being integrated x time distance between beats) for different metronome frequencies. The results showed, *first*, that the magnitude of integration periods significantly depends on a frequency of presented metronome beats. When the frequency of metronome beats was higher, the time interval in which the subjects integrated beats into an unit was shorter. *Second*, the elder adults integrated the information in the longer time interval then the younger ones. *Third*, the basic measure of integration periods was differentiated as related to subjects' level of cognitive abilities. These results suggest that the magnitude of temporal integration limit is not a constant, stable feature, but varies across a life span depending on mental content of information being integrated and on several individual factors.

Oral communications 2 - Neuropathology

THE ORIGIN OF Aß IN PLAQUES AND VESSELS IN AGING AND ALZHEIMER DISEASE H.M. Wisniewski*, J. Wegiel*, J. Frackowiak*, T. Wisniewski** *NYS Institute for Basic Research, 1050 Forest Hill Road, Staten Island, NY 10314, **Dept. of Pathology & Neurology, New York University Medical Center, 550 First Avenue, New York, NY

The question of where the protein making amyloid originates is of critical importance for both localized and systemic types of amyloidoses. However, difficulty in identifying the origin of the protein making amyloid fibrils lays in the fact that many of the amyloidogenic proteins are ubiquitous proteins made by many cells in the body with splicing variants of the precursor protein. Based on extensive studies of human and animal neuritic and diffuse plaques an vessels affected by amyloid deposits, we came to the conclusion that in the brain both neuronal and non-neuronal cells are the source of Aß deposits. The diffuse or benign plaques appear to be of neuronal origin. In contrast, the neuritic or malignant plaques are the product of microglia cells. Perivascular cells (in capillary) and smooth muscle cells (leptomeningeal vessels) are responsible for amyloid formation in amyloid angiopathy. In tissue culture the myocytes isolated from vessels affected by amyloid angiopathy produce fibrillar and non-fibrillar Aß deposits. The tissue culture model of Aß formation open a new avenue for development of therapeutic strategies for Alzheimer disease.

AMYLOID A β1-42 DOES NOT LEAD TO SENILE PLAQUES IN THE CANINE AGING MODEL OF ALZHEIMER'SDISEASE

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Alzheimer's disease (AD) is neuropathologically defined by the deposition of amyloid in the form of senile plaques, congophilic angiopathy and neurofibrillary tangles. The major constituent of senile plaques is amyloid β (A β), which also exists as a normal constituent of biological fluids called soluble A β (sA β). An early stage in the development of senile plaques are preamyloid (diffuse) lesions. These are A β immunoreactive areas, which are Congo Red and Thioflavin S negative and ultrastructurally are mainly non-fibrillar. We hypothesize that sA β can first aggregate into preamyloid deposits, followed by a gradual compaction and fibrillization over many years; only in this form is significant toxicity associated with the A β. Aged dogs can develop extensive preamyloid deposits and congophilic angiopathy; however, progression onto senile plaques is rare. We have biochemically extracted canine leptomeningeal A β and preamyloid, subjecting it to N-terminal sequencing and mass spectroscopy. Approximately 58% of leptomeningeal amyloid A β peptides extended to the valine which corresponds to amino acid 40 of A β (A β40) while the rest extended to the alanine that corresponds to residue 42 of A β(A β42). The majority of preamyloid consisted of A β42. These biochemical results were also verified immunohistochemically using antibodies specific for A β40 and A β42, with good correlation. This data suggests that longer A β peptides extending to residue 42 may be involved in promoting preamyloid deposits but are not sufficient, without the presence of other factors, for the further development of senile plaques. In cerebral and systemic amyloidoses amyloid deposits are invariantly associated with amyloid associated proteins. The presence of one of these, apolipoprotein E (apo E) was evaluated immunohistochemically and was found to be absent in canine preamyloid deposits. In AD, as in many of the systemic amyloidoses, it is unlikely that the exact length of the amyloid forming peptide is critical in the disease pathogenesis and the absence or presence of other factors, which function as "pathological chaperones", may be crucial for development of senile plaques.

APOLIPOPROTEINS E, J AND A-1 IN BRAIN AFTER COMPLETE CEREBRAL ISCHEMIA

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The distribution of apolipoproteins E, J and A-1 (Apo E, J and A-1) was investigated immunocytochemically in rats after 10 min complete cerebral ischemia (CCI) induced by *cardiac arrest*. Strong Apo E and weaker Apo J immunoreactivity was found extracellularly located close to the microvessels. These deposits appeared 3h after CCI and were present, but not in all the animals, at all times after CCI. In some rats, Apo E immunoreactivity was also found in small necrotic foci. Widespread, neuronal Apo E immunostaining appeared 6h after CCI. However, the strongest neuronal Apo E immunoreactivity was found 7 days after CCI in those neurons, most often observed in the CA1 hippocampal sector, exhibiting signs of ischemic cell damage. These ischemically damaged neurons displayed weaker immunoreactivity to Apo J. Our data show that mechanisms operating in ischemia are able to supply large amounts of Apo E and Apo J to the brain tissue and suggest involvement of both Apo E and Apo J in a complex series of events occurring in the ischemic brain. Perivascular deposits of Apo E/Apo J and Apo A-1 colocalized with amyloid ß-protein precursor epitopes in this model. Whether this phenomenon is limited to postischemic brian tissue, or can be encountered also in other pathological conditions will require further elaboration.

Alzheimer's Disease and Apolipoproteins

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Alzheimer's disease (AD) is the most common cause of dementia in the Western world. Neuropathologically AD is characterized by the deposition of amyloid in the form of senile plaques and congophilic angiopathy, as well as, neurofibrillary tangles. The major protein deposited in senile plaques is amyloid β(Aβ). The latter is a peptide of 39 to 44 amino acids, that is derived from a larger precursor: the β amyloid precursor protein (βPP). Some of the rare early onset forms of AD have been linked to mutations in the βPP gene or to an unknown locus on chromosome 14. However, the major risk factor for the most common forms of AD,sporadic and familial late-onset AD, is the presence of the apolipoprotein (apo) E4 allele. Prior to the linkage studies, the presence of apo E epitopes immunohistochemically within senile plaques led to the hypothesis that apo E can modulate amyloid fibril formation and acts as a "pathological chaperone". Consistent with this notion, we have shown *in vitro* that apo E, and apo E4 in particular, enhance Aβ fibril formation. Furthermore, our recent biochemical extractions from AD patient's plaque amyloid have found that a carboxyl fragment of apo E co-purifies with Aβ. Thrombin cleavage of apo E generates a similar carboxyl fragment, which *in vitro* can form amyloid-like fibrils which are Congo Red positive. These results suggest that senile plaques may contain two types of amyloid fibrils. AD can be viewed as a disease defined neuropathologically by the presence of several interacting proteins, which can adopt an amyloidogenic conformation.

RESEARCH UPDATE ON NEURONAL CEROID LIPOFUSCINOSIS

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Neuronal ceroid lipofuscinosis (NCL) are one of the most common progressive neurodegenerative diseases in infancy and children and are inherited mainly with an autosomal recessive trait. The infantile form is linked to chromosome 1p32 and the juvenile form is linked to chromosome 16p12. The linkage site for the late infantile (LINCL) and adult forms are not known. Clinicopathological data on heterogeneity obtained for different NCL forms (>500 cases): juvenile (48%), late infantile (42%), infantile (7%), and Kufs (3%) will be discussed. Subunit c of mitochondrial ATP synthase is a component of the lysosomal storage material in the brain and other tissues of various forms of NCL, except for the infantile (INCL) form. In searching for an easily available diagnostic test for NCL, using Western blot analysis, ELISA technique, and modified blotting procedure, we have found significantly higher levels of subunit c in the urine of LINCL and some JNCL patients [Wisniewski, et al. 1993; 1995, in press]. The latter technique provides results within hours, and may represent a new diagnostic screening test for individuals with NCL, especially LINCL and some JNCL cases.

The acute cell death via apoptosis induced by vincristine in the brain of rabbits

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Considerable amount of information is available on the cellular targets and mechanism of action of vincristine as the anticancer agent. However, little is known about the acute cell death mechanism the drug may induce in the nerve tissue. The aim of the study was to answer the question whether vincristine may induce such cell death in the brain. The young (two-week old) and adult (three-month old) rabbits were treated with single intraperitoneal injection of vincristine. Animals were sacrificed for the study 1, 2, 4, 6, 12, 24 hours and 7 days after the treatment. Samples of the brain were embedded in epon and examined in electron microscope. In both groups of animals the sequence of programmed cell death events was found. Initial step included condensation of chromatin and isolation of affected cells from their neighbours already 1, 2 and 4 hours after the treatment. Apoptotic bodies were most numerous 2 and 4 hours but sporadically found also 7 days following vincristine administration. Ingestion of the apoptotic bodies and the subsequent degradation of them by the lysosomal enzymes of the recipient cells were observed during the whole period of the study. Additionally in both groups of animals numerous degenerating "dark neurons" were found. Our results lead to the conclusion that vincristine is a potent inducer of apoptosis in the brain and that this type of cell death may play an important role in vincristine neurotoxicity.

Ultrastructural neuropathology of transgenic mice with human foamy virus is reminiscent of of subacute spongiform encephalopathies or AIDS-vacuolating myelopathy

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We report here the ultrastructural pathology of transgenic mice expressing the human foamy virus genome. Overall, the pathological changes were minimal and scattered; thus serial sectioning was necessary. Cerebellum was affected mostly. Membrane-bound vacuoles dominated the ultrastructural picture. All vacuoles were clearly derived from myelinated fibers. The myelin sheath which lined them was either apparently normal or composed of only a few layers; thus the latter was much thinner than normal myelin from axons of analogous diameter. Some vacuoles seemed apparently empty but others exhibited what seemed to be remnants of axons. The latter was shrunken and electron-dark. Numerous blebs and "curled" membrane fragments were also visible within vacuoles. Dystrophic neurites accumulated abnormal subcellar organelles and mitochondria. Some other peculiar changes deserves separate comments. Several cell processes intermingled between myelinated fibers contained abundant coated vesicles. We were unable to trace these processes to the cell bodies; thus their origin is uncertain. Strange curvilinear pits reminiscent of coated pits or early virus budds were also encountered. The last finding consists of paired thick tubules immersed within cytoplasm of very rare axons. These were reminiescent of longitudinally-sectioned walls of so called "test tubes" inclusions encountered in some virus disorders including AIDS. All these findings are reminiscent of early changes encountered in mice infected with the Fujisaki strain of Creutzfeldt-Virus disease and AIDS vacuolar myelopathy.

Aguzzi A: The foamy virus family. Molecular biology, epidemiology and neuropathology. *Biochim. Biophys. Acta* 1993; 1155: 1-24

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Oral communication 3 - Neuropharmacology II - Varia

STUDIES ON MECHANISMS INVOLVED IN SENSITIZATION TO EFFECTS OF COCAINE

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Senstization to cocaine effects is a well-known phenomenon which is a kind of "reversed tolerance" and which might be relevant both for addicting properties and for psychotic alterations after chronic treatment. Findings described in the literature suggest that sensitization is a complex phenomenon, mainly involving dopaminergic systems. Actions in the substantia nigra appear to be relevant for the development, in the striatum (both pre- and postsynaptic mechanisms) for the manifestation or expression of a sensitization.
In own studies, it was shown in the EEG that 10 mg/kg i.p. of cocaine induced a pattern suggesting activation of dopamine D_1 receptors, both after a single and repeated administration. A pattern in the EEG suggesting D_1 activation was also found after a single administration of 20 mg/kg of cocaine, whereas repeated administration induced a pattern suggesting additional activation of D_2 receptors. This pattern resembles that observed after a single administration of 30 mg/kg of cocaine. In additional behavioural experiments, it was found that part of the sensitization could be attributed to non-associative mechanisms, whereas another part was due to associative, conditioning mechanisms which were under control of external, conditioning stimuli. Both types of sensitization could not be attributed to alterations in extracellular striatal dopamine as measured by microdialysis in awake freely moving rats.
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CHANGES IN MOUSE BRAIN GABA METABOLISM DURING LORAZEPAM INDUCED ABSTINENCE SYNDROMES

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The possible changes in the metabolism of GABA at different brain regions of Lorazepam (LZ) dependent mice 24 h after withdrawal were studied and compared with those induced after acute and chronic administrations.

LZ (1mg kg^{-1}) was administered to male mice i.p. twice daily for 10 days, then doubling the dose during the next 10 days. The animals were divided into 2 groups, one used to determine the chronic effects, 2h after the last dose, while the other was used to investigate the effect of LZ withdrawal 24 h after the last dose.

In LZ dependent mice, behavioral changes were recorded using scores for hypermotility, muscle rigidity and seizures, and were found to be 40%, 50% and 20%, respectively. These symptoms were accompanied by a significant decrease in GABA and glutamate contents as well as aspartate aminotransferase (AST) activity in the cerebral cortex, thalamus hypothalamus and cerebellum, which might indicate decreased GABA biosynthesis during LZ withdrawal. On the other hand, acute and chronic administrations of LZ were associated with a decrease in glutamate accompanied by an increase in GABA content and AST activity while GABA aminotransferase activity was unchanged. This might indicate increased GABA synthesis from glutamate.

NEUROPHARMACOLOGY AND CHEMICAL ECOLOGY OF A NEUROTOXIC POLYPEPTIDE.

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In venoms, which are in nature injected into the circulation of a prey organism, polypeptides play the main pharmacological role as neurotoxins, cytolysins and enzymes. However, as shown by numerous studies, chemical interactions among organisms mediated by pheromones, kairomones and allomones are performed through low molecular weight, non-peptide and often volatile substances.

Our studies reveal that in a marine environment the same polypeptides may fulfill neurotoxic as well as message carrying roles owing to the high information content inherent in their structures and their solubility in water. We show that the mollusc-selective conotoxin (TxVIA) derived from the venom of a molluscivorous Conus snail demonstrates a unique "double duality":

1. On the cellular-subcellular level, and from a neuropharmacological point of view, it functions as both an agonist and an antagonist.
2. On the organismic-ecological level, and from the point of view of chemical ecology, if functions as both an allomone and kairomone.

THE NMDA RECEPTOR COMPLEX AND DEPRESSION

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Functional antagonists of the NMDA receptor complex are active in both preclinical behavioral paradigms sensitive to antidepressants (ADs) and in the chronic mild stress (CMS) animal model of depression. Chronic AD treatment induces adaptation of the NMDA receptor complex in naive rodents. Specifically, this adaptation is evident as a reduction in the potency of glycine to displace [^{3}H]5,7-dichlorokynurenic acid (5,7-DCKA) from the glycine recognition site and/or the reduction in the proportion of high affinity, glycine-displaceable [^{3}H]CGP-39653 binding to the glutamate recognition site of the NMDA receptor complex. We tested the hypothesis that animal models of depression would result in AD-reversible adaptation of the NMDA receptor complex. We now report that CMS results in the enhancement of the potency of glycine to displace [^{3}H]5,7-DCKA binding, which is reversible by chronic imipramine treatment. In addition, we tested the hypothesis that depression in humans is associated with NMDA receptor dysfunction. We examined frontal cortical samples from sucide victims matched with sudden death control subjects. We report that the potency of glycine to displace [^{3}H]5,7-DCKA binding was unchanged, while specific [^{3}H]CGP-39653 binding as well as the proportion of high affinity, glycine-displaceable [^{3}H]CGP-39653 binding were reduced in suicide victims. These data support the hypothesis that depression is associated with dysfunction of the NMDA receptor complex.

Electron-immunocytochemical localization of endothelial and neuronal isoforms of nitric oxide synthase in rat cerebral basilar artery

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Nitric oxide (NO) is a potent vasodilator, synthesized by nitric oxide synthase (NOS), a highly active enzyme in endothelial cells (eNOS, isoform type III) and neurons (nNOS, isoform type I). Both eNOS and nNOS are constitutive, agonist triggered and dependent on Ca^{2+}/calmodulin. NO acts as an endothelium-derived relaxing factor (EDRF) and as a non-adrenergic, non-cholinergic neurotransmitter in several parts of the central and peripheral/autonomic nervous systems. NO from cerebral endothelium and perivascular vasodilator nerves may therefore be involved in the endothelial and neural control of cerebral blood flow. We have examined the ultrastructural distribution of eNOS and nNOS immunoreactivity in perivascular nerves (axons) and vascular endothelial cells of the Wistar rat cerebral basilar artery, employing monoclonal antibodies to eNOS and nNOS in pre-embedding (PAP) and post-embedding (colloidal-gold) immunocytochemistry. Using the PAP method, cytoplasmic immunoreactivity to eNOS and nNOS was seen in subpopulations of both the endothelial cells (approx. 27.5% and 6.1%, respectively) and perivascular nerves (approx. 25.4% and 24.9%, respectively). The results indicate that both eNOS and nNOS are localized in different proportions of vascular endothelial cells but in similar proportions in perivascular nerves. Using the colloidal-gold method, eNOS and nNOS labelling signal dominated in the cytoplasm/axoplasm. In the n/eNOS-positive axon varicosities, the immunogold signal was sometimes observed within the lumen of small agranular synaptic vesicles (of 40-50nm). The present data extends our earlier electron-immunocytochemical observations using polyclonal nNOS antibody (Loesch A, Belai B & Burnstock G, 1994 J Neurocytol 23:49-59) to detect NOS in rat cerebrovascular endothelium and perivascular nerves.

SPECIFIC EFFECT OF NIMODIPINE ON CAT VISUAL NEURONS REVEALED BY SIMULTANEOUS RECORDINGS

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Controlled clinical trials demonstrated that nimodipine prevents form arterial vasospasm after subarachnoid hemorrhage. In spite of the well investigated cerebrovascular effects, no obvious EEG-alteration after administration of nimodipine in the cat was observed.

We recorded single or multiple units with an array of 8 microelectrodes in cat visual cortex. Light bars were swept at various speeds and orientations along trajectories to stimulate receptive fields (RF). In addition, on/off-stimuli were applied. Recordings were performed before, 5 and 30 min after i.v. administration of 1-2mg/kg nimodipine. The neuronal response was characterized by means of psths including maximum amplitude, orientation or direction selectivity, RF-width and visual latency.

As a rule psth-amplitudes were reduced after 5 and increased after 30 min. However, the effect of nimodipine on the amplitude could differ in simultaneously recorded neurons. The orientation selectivity was clearly diminished during the first control but restored after 30 min. The latency after stimulation with moving bars was prolonged after 5 and shortened after 30 min. On the contrary, RF width was first decreased. Latency following on/off stimulation was not altered by nimodipine. Direction properties were also regularly influenced by the drug although no systematic change was ascertained.

In conclusion, our results revealed specific effects of nimodipine on the response of cat visual neurons, which cannot be explained by vasodilation.

Poster sessions - Excitatory amino acids

EXCITATORY AMINO ACID ANTAGONISTS IN THE TREATMENT OF PESTICIDE INTOXICATION IN MICE

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Excitatory amino acid antagonists have been proposed to possess neuroprotective, anticonvulsant, antiischemic and myorelaxant properties. Possible beneficial effects of a competitive NMDA antagonist D-CPPene, a non-competitive NMDA antagonist MK-801 and a strychnine-insensitive glycine binding site antagonist felbamate, upon toxicity induced by systemic exposure to hydrocarbon insecticides were studied in mice. Experimental groups consisted of 6 - 8 mice, weighing 20 - 30 g. All the drugs were administered intraperitoneally. Hydrocarbon pesticides: dieldrin, α-endosulfane and lindane produced clonic - tonic convulsions and death of animals in a dose-dependent manner. D-CPPene, MK-801 and felbamate reduced the lethality of animals following the pesticide administration. D-CPPene (20 mg/kg, 30 min) produced more than 2, 3 and 10-fold increase in the lethal doses of dieldrin, α-endosulfane and lindane, respectively. Pretreatment with MK-801 (0.4 mg/kg, 30 min) lowered the lethality of mice treated with the pesticides with a similar potency. Felbamate (400 mg/kg, 45 min) elevated the lethal doses of α-endosulfane, lindane and dieldrin from 7.2 to 9.6, from 47.1 to 192.6 mg/kg and from 31.5 to >80 mg/kg, respectively. The excitatory amino acid antagonists blocked tonic convulsions induced by all the pesticides tested and elevated the threshold for clonic seizures evoked by dieldrin. It may be suggested that NMDA antagonists offer a novel possibility of the pesticide poisoning treatment.

INTERACTION OF EXCITATORY AMINO ACID ANTAGONISTS AND CALCIUM CHANNEL INHIBITORS IN THE ELECTROCONVULSIVE TEST IN MICE.

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A number of NMDA, AMPA/KA antagonists and calcium channel inhibitors (CCIs) were shown to potentiate the protective action of conventional antiepileptics against electroconvulsions in mice. However, some of these combinations resulted in profound adverse effects. Herein, we report on the interaction of GYKI 52466 (an AMPA/KA receptor antagonist) and LY 235959 (an NMDA receptor antagonist) with three CCIs (nifedipine, nicardipine and flunarizine) in the maximal electroshock-induced convulsions (ear-clip electrodes, 0.2 s stimulus duration, alternating current of 25 mA, tonic hindlimb extension as the endpoint) in mice. Nifedipine and nicardipine (up to 15 mg/kg) and flunarizine (up to 10 mg/kg) remained ineffective upon the convulsive threshold. Flunarizine (1.25-10 mg/kg), nicardipine (15 mg/kg) and nifedipine (15 mg/kg) potentiated the protective activity of GYKI 52466. Flunarizine (1.25-10 mg/kg), nicardipine (1.88-15 mg/kg) and nifedipine (0.47-15 mg/kg) enhanced the anticonvulsant action of LY 235959. The combined treatment, providing a 50% protection against electroconvulsions, resulted in a moderate motor impairment (glutamergic antagonists + nifedipine or nicardipine) but did not result in a worsening of the motor performance in the case of flunarizine. However, long-term-memory was not significantly impaired only in mice, receiving a combination of flunarizine and GYKI 52466. It can be concluded that some combinations of CCIs and excitatory amino acid antagonists may bear a therapeutic potential in the treatment of epilepsy.

EFFECTS OF APV CONTAINING ELVAX IMPLANTS UPON BASAL AND STIMULUS-EVOKED OF 2DG UPTAKE IN THE BRAIN CORTEX

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The effects of chronic blocking of NMDA receptor by APV upon basal and stimulus-evoked uptake of [^{14}C]-2-deoxyglucose in the brain cortex were investigated. For this purpose we prepared implants based on synthetic resin Elvax, which was soaked with 50mM APV solution in water. Elvax was cut to 60μm thick 2x2mm slices and implanted subdurally over parietal cortex of mice and 2DG experiment was done 7 days later to evaluate basal and stimulus-evoked activity.

APV released from Elvax blocked 20% of NMDA receptors as revealed using *in vitro* autoradiography. Measurements of 2DG-labelling intensity in brains of normal mice that received Elvax-APV implants revealed that the basal metabolic rate was unchanged, as compared to the same site in the control hemisphere and to the site distant from the implant. The stimulus-evoked activity was slightly decreased in cortical layer II/III under the implants and also in layer IV.

Nissl-counterstaining revealed moderate damage of the cortex in site of implantation, with the greatest effects in upper cortical layers. Glial proliferation was also observed. Histological changes were restricted and not observed at 1-2mm distance from implant.

PARTIAL BLOCKING OF NMDA RECEPTORS IN THE BARREL CORTEX IMPAIRS PLASTIC CHANGES INDUCED BY LEARNING AND DEAFFERENTATION

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The whisker barrel system in rodents is a useful model for studying brain plasticity. In adult mice cortical representations of mystacial vibrissae can be altered as a result of deafferentation or of sensory training. We have mapped, with 2-deoxyglucose (2DG) autoradiography, the representation of a row of whiskers spared during ablation of vibrissal follicles performed 7 days earlier and a representation of a row of whiskers stimulated in a short-lasting (3 days) classical conditioning training where whisker striking was paired with a tail shock. Following deafferentation, a large (70%) increase of the width of cortical representation of the spared row was found in all cortical layers. After classical conditioning, the enlargement (40%) of cortical representation of the "trained" row was observed in layers IV and IIIb. Both treatments were unilateral and the hemisphere ipsilateral to the treatment served as control. We examined if these plastic changes can be prevented by the action of NMDA receptor blocker, APV. Implants of thin sheets of slow release resin Elvax impregnated with APV were inserted subdurally posterior to the barrel field region immediately before surgery or a day before the beginning of behavioral training. The amount of APV released blocked about 20 % of NMDA receptors in the barrel field, as ascertained by [3H]MK801 binding autoradiography. The implants did not significantly affect the 2DG basal and stimulus-evoked uptake in control mice. In both experimental groups, 2-deoxyglucose mapping did not reveal plastic changes of cortical representations of either spared or trained row of vibrissae. In a control group, where APV acted upon already present plastic change (implants were done 7 days after ablation of vibrissal follicles) the enlargement of cortical representation was observed, as was found in animals without implants. We conclude that low level of NMDA receptor blocking did not affect significantly the cortical metabolism of signal transmission, but prevented the experience-dependent plastic changes of the body maps in SI cortex.

Some behavioural effects of GYKI 52466, a non-competitive AMPA receptor antagonist

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GYKI 52466 (|1-(4-aminophenyl)-4-methyl-7,8-methyl-enedioxy-5H-2,3-benzodiazepine|) has been described as a non-competitive AMPA (non-NMDA glutamate) receptor antagonist. In the present study some behavioural effects of GYKI 52466 were measured in rats and mice. For comparison, the NMDA receptor antagonist, CGP 37849 (DL-/E/-2-amino-4-methyl-5-phpsphono-3-pentenoic acid) was used.

GYKI 52466 reduced the locomotor activity in normal rats and mice without evoking any symptoms of behavioural stimulation. The CGP 37849-induced hyperlocomotion was increased by GYKI 52446. The akinesia in reserpinized rats was not affected by either drug studied. The antiakinetic effect of L-DOPA was increased by CGP 37849, but not by GYKI 52466. GYKI 52466 did not change the catalepsy induced by haloperidol or fluphenazine, while CGP 37849 antagonized it. GYKI 52466 was inactive in the forced swimming test, but increased the antidepressant effect of CGP 37849. The muscle tone was reduced by CGP 37849, but not by GYKI 52466. The obtained results indicate that the AMPA receptor antagonist differs in its neuropharmacological profile from the NMDA receptor antagonist. In some tests, a positive interaction between the two drugs was observed.

THE ENHANCEMENT OF NORADRENALINE INDUCED CYCLIC AMP ACCUMULATION IN RAT BRAIN BY STIMULATION OF METABOTROPIC GLUTAMATE RECEPTORS OCCURS IN CEREBRAL CORTICAL SLICES AND IS MEDIATED BY ENDOGENOUS ADENOSINE.

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The effect of several metabotropic glutamate receptor agonists and antagonists on noradrenaline (NA)-stimulated [^{3}H]-cyclic AMP accumulation was investigated in rat cerebral cortical slices and in glial and neuronal cell cultures. (2S,3S,4S)-α-(Carboxycyclo-propyl)glycine (L-CCGI), 1-Aminocyclo-pentane-1S, 3R-dicarboxylate (1S,3R-ACPD), ibotenate (IBO) and (RS)-4-carboxy-3-hydroxyphenylglycine (CHPG) elicited a concentration-dependent enhancement of NA-stimulated [^{3}H]-cyclic AMP accumulation, with EC_{50} values of 2.5 ± 0.11, 42 ± 1.3, 97.8 ± 2.1 and 157 ± 13.4 μM, respectively. (S)-3-carboxy-4-hydroxyphenylglycine (3C4HPG) and (S)-4-carboxy-3-hydroxyphenyl-glycine (4C3HPG) produced a biphasic effect, at concentrations up to 100 and 500 μM, respectively, they significantly enhanced the action of NA (100 μM), at 1mM concentration both compounds as well as α-methyl-4-carboxyphenylglycine (MCPG) produced a significant inhibition of NA-stimulated cyclic AMP accumulation. L-AP3 inhibited the 1S,3R-ACPD (100 μM)-induced enhancement of the action of NA (100 μM) on [^{3}H]-cyclic AMP accumulation in a biphasic manner with an IC_{50} of 4.5 μM for the high affinity site, which represented 65% of the total and an IC_{50} of 283 μM for the low affinity site. Neither the protein kinase C inhibitor - staurosporine (10 μM) nor thapsigargin (1 μM), which depletes IP3 sensitive calcium stores, inhibited significantly the 1S,3R-ACPD (100 μM)-induced enhancement of the action of NA (100 μM) on [^{3}H]-cyclic AMP accumulation. Adenosine deaminase (0.5 U/ml) abolished both the 1S,3R-ACPD (100 μM)-induced [^{3}H]-cyclic AMP accumulation and the synergistic interaction of this compound with NA (100 μM). In glial cell cultures 1S,3R-ACPD (100 μM) inhibited the NA (100 μM)-induced [^{3}H]-cyclic AMP accumulation, while in neuronal cell cultures it had no effect. These results indicate that he enhancement of the action of NA on cyclic AMP accumulation by 1S,3R-ACPD occurs in cerebral cortical slices and is mediated via receptors which are blocked with high affinity by L-AP3, the endogenous adenosine is involved in the interaction.

IMPACT OF SELECTIVE ANTAGONIST OF 5-HT_{1A} RECEPTORS ON BIOCHEMICAL AND BEHAVIORAL EFFECTS OF NON-COMPETITIVE NMDA ANTAGONIST MK-801

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There are several reports indicating that drugs operating via 5-HT_{1A} receptors might have putative therapeutic potentials in treatment of side- effects evoked by competitive and non-competitive NMDA antagonists. Above observations were based on non-selective 5-HT_{1A} antagonists like NAN-190. Availability of highly selective 5-HT_{1A} antagonists WAY 100135 inclined us to reinvestigate above observations. We analyzed the impact of WAY100135 (10 and 20 mg/kg) on the effects of MK-801 (0.4 mg/kg) in three experimental paradigms a) MK-801 evoked enhancement of dopamine release in prefrontal cortex of freely moving rats - brain microdialisis; b) MK-801 evoked disruption of rat sensorimotor gating - prepulse induced inhibition of the acoustic startle response and c) MK-801 induced locomotor hyperactivity. It was found that enhancement of dopamine release evoked by MK-801 in the rat prefrontal cortex was antagonized by WAY 100135, while WAY100135 failed to alter the MK-801 evoked disruption of sensorimotor gating and MK-801 evoked enhancement of the locomotor activity. It is concluded that selective blockade of 5-HT_{1A} receptors has limited value as a therapeutic strategy which may antagonize psychotomimetic effects of non-competitive NMDA antagonists.

THE SUBSENSITIVITY OF STRIATAL GLUTAMATE RECEPTORS INDUCED BY CHRONIC HALOPERIDOL IN RATS

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The aim of the present study was to investigate the influence of chronic treatment with haloperidol on the contralateral head turns and rotations induced by intrastriatal agonists of NMDA and non-NMDA receptors in rats. N-methyl-D-aspartate (NMDA, 500 ng/0.5 µl), α-amino-3-hydroxy-5-methyl-4-isoxasole-propionic acid (AMPA, 1000 ng/ 0.5 µl) or kainic acid (50 ng/0.5 µl), injected into the intermediate and caudal parts of the caudate-putamen, induced contralateral head turns and rotations. Haloperidol was given to animals in a dose of ca. 1 mg/kg/day in drinking water for 6 weeks. On day 5 of withdrawal, haloperidol decreased the number of contralateral head turns, but did not significantly influence the contralateral rotations induced by NMDA, AMPA and kainic acid. At the same time, haloperidol enhanced the stereotypy induced by apomorphine (0.25 mg/kg sc). The present results suggest that chronic treatment with haloperidol induces supersensitivity to dopamine and subsensitivity of striatal NMDA and non-NMDA receptors.

OVEREXPRESSION OF CREM/ICER mRNA IN THE RAT BRAIN FOLLOWING TREATMENT WITH NMDA RECEPTOR ANTAGONISTS

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CREM/ICER (cAMP responsive element modulator/inducible cAMP early repressor) is a transcription factor gene encoding a variety of gene regulators (both positive and negative) generated through alternative splicing, as well as differential use of two promoters. Recently, it has been shown that ICER protein products play a pivotal role in regulation of diurnal rhythm of melatonin production by pineal gland. It has been also noted that -adrenergic receptors are driving ICER gene expression in this physiological reponse. We have observed elevated CREM/ICER mRNA levels in the rat visual cortex following visual exposure, subsequent to visual deprivation. This last response was concomittant with elevated expression of other genes coding for transcription factors, namely, *c-fos, jun B* and *zif 268*. Since expression of all of the three latter genes is known to be NMDA receptor dependent, we have also investigated effects of MK-801 (1 mg/kg) as well as CGP 40116 (10 mg/kg), two different NMDA receptor antagonists on CREM/ICER gene expression. Surprisingly, both compounds provoked dramatic accumulation of CREM/ICER mRNAs. This effect was observed by northern blot analysis, even in the absence of visual stimulus. At present we are employing in situ hybridizatoion to get further insight into neuranatomical localization of the effect.

KAINATE-EVOKED SECONDARY GENE EXPRESSION IN THE RAT CORTEX

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Kainate, an agonist of excitatory amino acids receptors, is known to evoke sustained enhancement of neuronal activity. Since kainate treatment induces elevated gene expression as well, it is often used as model system of neuronal genomic response to activation of EAA receptors. Importance of this model system is further underscored by well defined role of these receptors in neuronal plasticity. However, most of the studies on kainate-elevated gene expression have dealt with transcription factors like c-fos, c-jun, their cognates, and zif/268, as well as other immediate early genes. Unfortunately, very little is known about target genes for the transcription factors, whose expression should comprise a secondary event in neuronal response to kainate. Recently, we defined secondary nature of three genes: secretogranin II, clathrin heavy chain and hsc 70, whose mRNA accumulates in the rat hippocampus following kainate treatment (Konopka et al., Neurosci. Lett., in press). In order, to get further insight in their expression, we decided to analyze, with an aid of isotopic and non-isotopic in situ hybridization, as well as northern blotting, their mRNA levels in other cortical areas. We have found that increased secretogranin II mRNA level can be observed clearly in limbic cortex, and in parieto-occipital neocortex. The clathrin heavy chain mRNA was accumulated in limbic cortex but not neocortex, while hsc 70 gene expression was found to be only weakly elevated in the both cortical areas. These results point to differential regulation of expression of each of the three genes following kainate treatment.

Immunolocalization of AP-1- and CRE-DNA binding proteins in the rat brain

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Short regulatory DNA sequences named AP-1 and CRE can be bound by different proteins acting as transcription factors. These include AP-1 dimers (composed of various Fos and Jun proteins) as well as CREB/ATF/CREM proteins. Recently a group of proteins called Maf was also shown to bind to AP-1 and CRE elements. Since both CRE and AP-1 sites play a critical role in neuronal plasticity including learning and memory phenomena, we decided to investigate immunocytochemical localization of AP-1 proteins (c-Fos, Fos B, Fra-1, Fra-2, c-Jun, Jun B, Jun D), CREM as well as v-Maf and Maf K proteins in the brains of naive rats as well as animals treated with excitatory amino acids' receptor agonist as well as proconvulsant and neurotoxic agent - kainate (10 mg/kg, pH 7.0). Rats were sacrificed 0h, 6h, 24h and 72h following the treatment. These time points were selected as correlating either with the period of intense seizures (6h) or with widespread neurodegeneration (72h). We found that all the proteins investigated show specific temporal and spatial (mainly nuclear) expression patterns. The differences in observed patterns of induction suggest differential involvement of the transcription factors' proteins under study in plasticity/ neurodegeneration phenomena.

THE SUBSENSITIVITY OF STRIATAL AMPA RECEPTORS, INDUCED BY CHRONIC HALOPERIDOL IN RATS: AN AUTORADIOGRAPHIC ANALYSIS

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The aim of the present study was to assess the influence of chronic treatment with haloperidol on the density of D2 dopamine, NMDA and AMPA receptors in the caudate-putamen of rats by an autoradiographic analysis. Haloperidol was given to animals in a dose of ca. 1 mg/kg/day in drinking water for 6 weeks or 3 months. On day 5 of withdrawal, the rats were killed by decapitation. The following ligands were used: [3H]spiperone for labelling D2 dopamine receptors, [3H]CGP 39653 and [3H]MK-801 (dizocilpine) - for recognition and phencyclidine binding sites of the NMDA receptor complex, respectively, and [3H]AMPA - for AMPA receptors. 6-week and 3-month treatments of rats with haloperidol significantly increased the density of D2 dopamine receptors in the rostral and intermediate-caudal regions of the caudate-putamen. The density of AMPA receptors in the intermediate-caudal caudate-putamen was significantly decreased after 3-month haloperidol administration. In contrast, chronic haloperidol did not influence the binding of [3H]CGP 39653 or [3H]MK-801 to the striatal tissue. The present results suggest that chronic treatment with haloperidol induces supersensitivity of D2 dopamine receptors and subsensitivity of AMPA receptors in the caudate-putamen.

Poster sessions - Neurochemistry

CHANGES IN THE LEVEL OF -SH GROUPS IN BRAIN UNDER LEAD-TOXICITY CONDITIONS.

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In lead neurotoxicology an important issue relates to how lead interferes with chemical neurotransmission. We supported postulate of other authors that Pb^{2+}/Ca^{2+} interactions might play an important role in the toxicity of lead in neurotransmission, but our investigations suggest the existance of several mechanism of lead toxicity related to neurotransmitter transport. Our erlier data have shown that disturbances in the energetic metabolism and in activity of Na^{+}-K^{+}-ATPase in the Pb toxicity can affect on neurotransmission. Now we investigated effect of Pb2+ on -SH groups level in the homogenates and synaptosomes obtained from rat brains. Two models of poisoning were used: 1. 200 mg $Pb(CH_3COO)_2$/l drinking water was given to 3 weeks-old rats for 3 mounths (chronic model). 2. 15 mg $Pb(CH_3COO)_2$/kg b.w. was injected intraperitoneally by 5 days into rats weighting approx. 200g (acute model). Synaptosomes were isolated from hemispheres according to Booth and Clark (Biochem. J.,1978,176,365) -SH groups level were measured according to the method of Sedlak et all, (Anal. Biochem. 1968, 25, 192).

Our data indicated: the lead caused statistically significant decrease of total and protein -SH groups level in synaptosomes by about 10% of control values in both chronic and acute models of toxicity, but has no effect on nonprotein -SH groups. In homogenates from whole brains obtained from Pb^{2+} poisoned animals, levels of total and protein -SH groups decrease by about 15% in acute model and about 10% in chronic model. Level of nonprotein -SH groups was practically not change. Decrease of protein -SH groups level by lead can influence activity of same enzymes and receptors, what in turn may cause disturbances in neurotransmitters transport.

LEAD AND BLOOD-BRAIN BARRIER IN ADULT RATS

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Symptoms of lead toxicosis in immature brain develop in severe form, what is connected with less effective blood-brain barrier (BBB). It is thought that parallel to the developing cerebrall microvasculature the resistance to the toxic substances increases and mature blood-brain barrier is a sufficient protection against toxic lead effects.

The study was performed to determine whether prolonged drinking of lead-containing water affects some properties of brain microvasculature in adult rats. 21-days-old Wistar rats received lead acetate in concentration 200mg/L in drinking water for 3 months *ad libitum*. After that time the horseradish peroxidase was used to estimate the BBB condition. We noted increased permeability to the marker in light- and electron microscopic studies, evidenced by enhanced transendothelial pinocytosis as well as by opening of interendothelial tight junctions, causing spread of the tracer to the extracellular space of neuropil. Numerous phagocytizing cells of pericytes origin with numerous phagolysosomes filled in with the HRP-reaction product were observed. These cells are mobilized in the CNS under influx of pathogenic factors and play an important role in the intoxicative processes.

The blood pressures in control and lead-treated groups were recorded, showing slight but signifficant increase in rats after lead exposure.It is possible that hipertension might be at least one of the reasons of observed disturbances in BBBcondition.Estimation of lead level revealed its signifficant accumulation in capillaries and synaptosomal fractions. It has to be remarked that lead enters the brain of not only young but also adult rats, disturbing BBB integrity and as the cosequence we observed changes in synaptosomal neurotransmission and perturbations in cell energetic processes.

ALTERATIONS OF $GABA_B$ BINDING CAUSED BY CHRONIC LEAD ADMINISTRATION.

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The mechanisms of lead toxicity to CNS are not clear. It was postulated that Pb^{2+}/Ca^{2+} interactions might play an important role in toxicity of Pb^{2+} in neurotransmission process. Our investigations sugested the existance of several mechanism of lead toxicity related to the individual neurotransmitter. In this work the effect of lead on $GABA_B$ binding was studied. The chronic model of poisoning was used; 200 mg $Pb(CH_3COO)_2$/l drinking water was given to 3-week old rats for 3 months. The mechanism of the γ-aminoacid receptor ($GABA_B$), one of the subtypes of GABA receptors was examined using crude membrane fraction (P_2) obtained from the rat brain according to Okmari et al (J. Neurochem. 1990,54,80). Chronic lead treatment produced specyfic receptors effects on binding GABA. Our data indicated that lead reduced $GABA_B$ receptor affinity (K_D) to GABA by about 25%. Also density (B_{max}) was changed. Pb^{2+} treatment increased density more than 20%. Displacement curve of binding [^{3}H] GABA after lead treatment was evident. The effect of lead on activity of $GABA_B$ receptor can be partially responsible for the observed erlier changes in GABA neurotransmission in brain.

PROGRESSIVE ALTERATION OF THE GABA/Cl^- CHANNEL PROPERTIES AND FUNCTION DURING REPERFUSION TIME IN THE HIPPCAMPUS AND THE BRAIN CORTEX.

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Properties and function of chloride channels were investigated by determination of association and dissociation kinetics of specific chloride (Cl^-) channel ligand [^{35}S]tert-butylbicyclophosphorothionate (TBPS). These studies were carried on in the absence and the presence of $GABA_A$ agonist (muscimol) using synaptic plasma membrane (SPM) from the hippocampus and the cerebral cortex isolated 4, 30 and 60 days after 5 min of bilateral occlusion of both common carotid arteries in gerbils. Furthemore, muscimol (20-100μM) stimulated $^{36}Cl^-$ uptake into synaptoneurosomes was determined. It was found that the half-life of fast phase of [^{35}S]TBPS dissociation which corresponds to an opening time of receptor-dependent Cl^- channel, was significantly decreased in the hippocampal SPM 4 days after arteries occlusion in the presence of muscimol. However, 30 and 60 days after ischemia, in spite of this modification of Cl^- channel, the half-life of fast phase of [^{35}S]TBPS dissociation was significantly lower in the brain cortex SPM, in the presence and absence of muscimol. These results suggest a decrease of opening time also the other Cl^- channels, not connected with $GABA_A$ receptor. Moreover the analysis of biphasic TBPS dissociation indicates a lowering number of Cl- channel "in open" state in brain cortex and hippocampus 60 days after ischemia. The significant lowering of GABA-activated Cl^- uptake in the presence of 35-50μM muscimol was observed 30 and 60 days after ischemia. Among processes which occur during ischemia and which potentially may induce modification of the Cl^- channels properties and function, the action of unsaturated fatty acids, peroxides and pH were taken into consideration. It was found that all of them modified the GABA-operated chloride channel function. Our results demonstrate that the properties and function of Cl^- channels were progressivelly affected by reperfusion injury. These alterations may be responsible for the lower hyperpolarization ability of $GABA_A$ receptor complex.

The subcellular localization of latent ribonucleases in different areas of rat brain.

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It is general agreement that in the animal tissues the neutral ribonuclease is bound to the endogenous protein, SH dependent inhibitor (SHI) as the substantial rise of activity take place after blocking of 100% SH groups. However we found in rat liver and brain the existence of two different latent ribonucleases (R) i.e. two complexes of R with SHI: 1) (known till now) LNR (i.e. latent neutral R) with optimum pH at 7,8 almost independent on rise of ionic strength and 2) (we found it) LMAR (i.e. latent moderately acid R) with optimum pH at 6O; it needs the elevation of ionic strength (to 0,14 M or more) to be active. From the homogenates of brain areas (cortex C, hipocampus H, midbrain M, thalamus and hypothalamus TH, cerebellum CE, medulla oblongata MO) subcellular fractions were isolated including purified nuclear, mitochondrial, synaptosomal and cytosolic. LMAR and LNR activities are the maximal in H and minimal in MO. Although 50% or more LMAR and LNR was found in cytosol the biggest "enrichment" of them was invariably found in the cell nuclei from all studied brain areas. Nuclear LMAR dominates over LNR in H and CE, whereas in TH and MO the opposite is true. The role of LMAR and LNR in RNA metabolism in brain (also in pathology) is discussed.

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ACETYL-CoA AND ACETYLCHOLINE METABOLISM IN BRAIN COMPARTMENTS OF THIAMINE-DEFICIENT RATS.

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One may assume that neuronal and glial compartments of the brain may display different vulnerability to pyrithiamine (PT) evoked thiamine deficiency. In PT-encephalopatic rats, activities of pyruvate and ketoglutarate dehydrogenases in synaptosomes were 10 and 40%, respectively lower than in controls. In PT-whole brain mitochondria these activities were diminshed for 30 and 40%, respectively. No changes in activities of carnitine acetyltransferase, choline acetyltransferase and ATP- citrate lyase were found. Pyruvate utilization and citrate accumulation in K-depolarized, Ca-activated PT synaptosomes were decreased for 25 and 45%, respectively, while oxoglutarate and lactate accumulations were increased by about 50%. Acetyl-CoA contents in whole PT terminals and in their mitochondrial and cytoplasmic compartmens were decreased for about 30%, but acetylcholine (ACh) synthesis was not impaired. On the other hand, in the absence of Ca, no changes in acetyl-CoA but 70% rise of resting ACh synthesis was observed in PT nerve terminals. Accordingly, Ca caused 120% rise in ACh synthesis in controls but only 20% in PT-s. Whole brain PT-mitochondria utilized pyruvate and accumulated citrate 20-30% slower than controls. However, their content of acetyl-CoA was depressed as much as 60%. The overall level of CoA remained unchanged. These data indicate that main defect of acetyl-CoA metabolism in thiamine deficiency is located rather in glial and pericarional mitochondia than in nerve endings. The excessive, Ca-independent release of ACh due to energy deficits may cause both seizures and transmitter depletion in brains of PT thiamine-deficient animals.

Okadaic acid as a probe for regulation of Mg - dependent Ca^{2+} -ATPase activity in synaptosomal membranes of rat brain.

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The phosphorylation processes mediated by protein kinases seem to be responsible for regulation in vitro of Mg-dependent Ca^{2+}-ATPase activity. In our study we tested the hypothesis that the dephosphorylation of enzyme by specific phosphoprotein phosphatases in vitro also modulate its activity. The experiments were performed on synaptosomal membranes from selected regions of rat brain - cortex and cerebellum. As an inhibitor of endogenous phosphatases PP2A and PP1 we used okadaic acid (OA), 10 nM and 1 μM, respectively. In standard conditions 10 nM OA increased the Mg^{2+}/Ca^{2+}-ATPase activity only in cortex. In the presence of 1 μM OA, the activity increased 2 times in both of examined regions. After addition of 1 μM PMA (activator of kinase C), the significant increase of basal activities were observed (50% in cortex and 270% in cerebellum). 1 μM OA enhanced the Mg^{2+}/Ca^{2+}-ATPase activity in both of regions, and 10 nM OA only in cerebellum. With 5 μM cAMP (an activator of kinase A) the cerebellar activity increased 2 times. The effect of 10 nM OA was similar in both of regions (135%). In cortex 1 μM OA caused further alteration of activity. These preliminary reports indicate that reversible phosphorylation could represent the important mechanism of regulation of Mg^{2+}/Ca^{2+}-ATPase activity, and this process in rat brain could be region-dependent.

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DEVELOPMENTAL CHANGES in BRAIN RIBOSOMAL PROTEINS PHOSPHORYLATION /STUDIES in VIVO and in VITRO/.

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In eucaryotic cells only a few of the 70-80 ribosomal proteins undergo phosphorylation, although, under certain conditions other proteins may become phosphorylated.

So far, little is known about the ontogeny of the ribosomal proteins phosphorylation in the brain.

Thus it was interesting to analyse the changes in ribosomal proteins phosphorylation during ontogenesis of chick brain, and to relate these changes to the activity of some soluble protein kinases.

The studies were perfomed on the brains of chick embryos and chickens ranging in the age between 5 and 150 days.

The level of in vivo and in vitro phosphorylation was measured as a transfer of pmoles of labelled phosphate into proteins from radioactiv orthophosphate and ATP respectively.

The intensity of ribosomal proteins phosphorylation decreased during development of chick embryo.
In experiments in vitro the phosphorylation increased during embryogenesis, and reached the highest level on 15 day, subsequently on 21 day /hatch/ it sharply decreased, and remaining on the unchanged level until 150 day.

Some qualitative differences between the proteins modified in vivo and in vitro were observed.

ANTIOXIDANT ENZYME ACTIVITIES IN DIFFERENT BRAIN AREAS OF THE NEUROLOGICAL MUTANT - PT RABBIT.

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The paralytical tremor rabbit, genetical symbol "pt" is a neurological mutant with hypomielinization and general neuron damage.
Cu, Zn - superoxide dismutase, Mn-superoxide dismutase, catalase, peroxidase, glutathione peroxidase and glutathione reductase activities were assayed in the brains of genetically selected neurological mutant rabbits "pt" and their controls.
Antioxidant enzyme levels were different in three brain regions: brain hemispheres, cerebellum and brainstem.
In brain hemispheres and cerebellum of "pt" rabbits Mn-SOD and Cu, Zn-SOD activities were elevated.
Catalase activity in brain hemispheres and peroxidase activity in the brainstem of "pt" rabbits were reduced.
It was also noticed, that in the "pt" rabbit the ratio CAT/Cu, Zn-SOD was lower by 20% in the brain hemispheres and by 13% in the cerebellum and the ratio POX/Cu, Zn-SOD was lower by 31,8% in the brainstem.
The overexpresion of Cu, Zn-SOD was found in the some human diseases of nervous system and indicated that deviations from the physiological activity ratio of antioxidant enzymes have been speculated to be one of the reasons of the observed brain damage in "pt" rabbits.

The chronic electroconvulsive treatment impact on the nitric oxide synthesis in a rat cortex synaptosomes

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Chronic electroconvulsive treatment (ECT) is widely used as an animal model mimicking convulsive disorders in man. An additional interest in ECT emerged from its antidepressive effect, however currently the extensive usage of ECT is discouraged. After chronic ECT (8 procedures or more) changes are seen in the signal transmission in the central nervous system, including alterations at the receptors and second messenger levels.

The goal of our study was to search for an impact of the ECT on the activity of a "constitutive", type I synthase of nitric oxide, a neurotransmitter stimulating cGMP synthesis and thus modulating signal transduction in neurons.

A sensitive spectrophotometric fluorimetry method was used for measurements of nitrate accumulation in cortex synaptosome preparations. Unstimulated cortex synaptosomes showed virtually the same, slow accumulation of nitrates (4.1 vs. 4.5 pM/mg of protein per hour) in the treated and the control group. Depolarization of synaptosomes with 10 mM KCl induced 6 fold increase in nitrate accumulation in the ECT treated group and 4.5 increase in the control. No differences between the groups were observed after L-Glutamate (1 μM), producing 3 fold increase of NO synthesis. An inhibitor of protein tyrosine phosphatase; vanadium hydroxyperoxide (pervanadate) produced a dose dependent (30 to 70 μM) increase in nitrate accumulation in both groups. However the maximal NO synthesis rate after pervanadate was 30% greater in the ECT treated group the difference was significant statistically, and stimulated levels were more than 10 fold greater then the basic ones.

The results indicate that chronic ECT induces an increase in NO synthesis in cortical nerve endings and that protein tyrosine phosphorylation seems to modulate potently the activity of the NO synthesis in CNS.

N-METHYL-D-ASPARTATE RECEPTORS AND DOPAMINE RELEASE IN EXPERIMENTAL HEPATIC ENCEPHALOPATHY

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Glutaminergic neurotransmitter systems have been thought to play an important role in the pathomechanism of hepatic encephalopathy (HE), a syndrome accompanied by disturbances in motor functions regulated by the basal ganglia. Dopamine (DA) release in the striatum is enhanced by the ionotropic glutamate receptors, in particular, by those of the N-methyl-D-aspartate (NMDA) class. We now evoked HE in male Wistar rats with intraperitoneal injections of a hepatotoxin, thioacetamide, 300 mg/kg on three successive days. The release of labeled preloaded DA from frontal cortical and striatal slices in vitro was enhanced by NMDA. The enhancement of DA release from striatal slices was reduced to about one half in HE. In frontal cortical slices HE did not affect the NMDA-stimulated DA release. In cortical synaptic membranes the NMDA-displaceable binding of [^{3}H]glutamate to the agonist site in the NMDA receptor complex was doubled in HE but the binding of dizocilpine (MK-801) and N-[1(2-thienyl)cyclohexyl]piperidine (TCP) to their sites in the NMDA-receptor-governed ion channel was not altered. The results were similar with hippocampal membranes. In the cortex dizocilpine binding was enhanced by glutamate and glycine more in HE rats than in controls. In striatal membranes the NMDA-displaceable binding of glutamate was not significantly altered in HE but the binding of both dizocilpine and TCP was markedly diminished. In all cases the maximal binding capacity B_{max} was the parameter affected most. The function of NMDA receptor complex in the striatum is thus compromised in HE in spite of the compensatory changes in the binding of glutamate to the agonist recognition site.

ENHANCEMENT OF [^{3}H]D-ASPARTATE RELEASE DURING ISCHEMIA LIKE CONDITIONS IN RAT HIPPOCAMPAL SLICES: SOURCE OF EXCITATORY AMINO ACIDS.

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Ischemic neuronal injury is caused in part by the accumulation of excitatory amino acids (EAA) in synaptic cleft. EAAs can be released into extracellular space from synaptic vesicles, metabolic pool of neurones and from cytoplasm of glial cells. The importance of these particular sources in ischemia - stimulated release is still under discussion. In this studies the release of non-metabolised analogue of glutamate: [^{3}H] D-aspartate ([^{3}H]D-asp.), loaded into 500 μm slices of rat hippocampus was investigated. The efflux of EAA was measured during anoxic - aglycemic (ischemic) incubation and in absence or presence of 65 mM KCl. To determine the pool from which [^{3}H] D-asp. is released we have used inhibitors of Na^+-dependent transporter of aminoacids: L-trans-pyrrolidine-2,4-dicarboxylic acid (PDC), sodium channel bloker tetrodotoxin (TTX) and furosemide which prevent astrocytes swelling. It is shown, that, upon KCl stimulation in normoxic condition, which enhances the release about five times, up to 40% of amino acids are released from synaptic vesicles (TTX and sodium dependency). In addition, nearly 40% can be transferred to the extracellular space by the reversion of Na^+-dependent transporter situated in the plasma membranes of both neuronal and glial cells. Simultaneous inhibition of these two mechanism reduces total release of EAA about 60 %. Ischemia does not change the basal EAA release, however when applied together with high K^+ concentration, increases it markedly. Moreover under isotonic conditions furosemide diminish [^{3}H]D-asp. release up to 50%. Additional 20 % can be blocked by PDC treatment.

Our data suggest, that EAAs accumulated in synaptic cleft during ischemia are mainly released from either cytosol of neurones or (and) glial cells with the involvement of the two major mechanisms. First, the mechanical stretching of astrocytes plasma membranes which stimulates EAA efflux mediated by recently reported stretching receptors activation. Second, disturbed ion homeostasis during ischemia would cause the reversion of Na^+-dependent amino acids transporter, shifting it toward the extracellular space.

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AGONIST-ACTIVATED Ca^{2+} AND Co^{2+} INFLUX THROUGH DIVALENT CATION-PERMEABLE KAINATE RECEPTORS

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Stimulation of kainate (KA) receptors may trigger diverse mechanisms of Ca^{2+}entry to neurons, including activation of voltage-sensitive calcium channels, reverse mode Na^+/Ca^{2+} exchange mechanisms and indirect activation of NMDA receptors. Recent reports indicate that the other subtype of non-NMDA glutamatergic receptors, AMPA receptors, may be permeable to Ca^{2+}. It has been also suggested that activation of KA receptors may induce Ca^{2+} and Co^{2+} influx into neurons through nonselective channels permeable also to Mn^{2+}. The aim of this study was to examine influx of divalent cations in the rabbit hippocampus evoked by stimulation of KA receptors. Our experiments with microdialysis of the rabbit hippocampus in vivo showed that application via dialysis probe of 1 mM KA and 1 mM domoic acid induces a 30% decrease in extracellular calcium concentration. Approximately 1/3 of KA-evoked extracellular Ca^{2+} decrease, that appears to be insensitive to nimodipine, amiloride derivatives and NMDA antagonists, may be ascribed to Ca^{2+} influx via KA receptor - operated ionic channels. This assumption is supported by a pattern of Co^{2+} uptake in rabbit hippocampal slices incubated in the presence of 0.25 mM KA and 0.1 mM domoate and AMPA. KA induced Co^{2+} uptake by all the pyramidal cells and by neurons in dentate gyrus (DG), domoic acid caused uptake of Co^{2+} in CA1, while AMPA stimulated Co^{2+} uptake mainly in CA1, CA2 and partially in DG. Our data suggest that in the rabbit hippocampus KA receptors operate at least two groups of ionic channels that are permeable to Ca^{2+} and other divalent cations.

$^{45}Ca^{2+}$ MOBILIZATION IN RAT HIPPOCAMPUS IN VIVO: NMDA RECEPTOR DEPENDENCE AND INTRACELLULAR MECHANISMS

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Recently we have noticed that in vivo application of N-methyl-D-aspartate (NMDA) via microdialysis probe to the adult rat, but not rabbit hippocampus, induces a huge release of $^{45}Ca^{2+}$ from the prelabeled tissue to dialysates. The aim of present study was to establish mechanism of $^{45}Ca^{2+}$ release in the rat hippocampus, its dependence upon NMDA receptors and Na^+/Ca^{2+} exchanger in the plasma membrane, and to characterize intracellular Ca^{2+} stores that contribute to the NMDA induced $^{45}Ca^{2+}$ release. Microdialysis experiments demonstrated dependence of $^{45}Ca^{2+}$ release on NMDA concentration in the dialysis medium in a range of 1 mM to 5 mM, and its complete prevention by 10 μM MK-801, thus indicating the role of NMDA receptors in mediating $^{45}Ca^{2+}$release. 5-(N,N-dimethyl)-amiloride (0.5 mM) strongly inhibited this effect, which suggests that Na^+/Ca^{2+} exchange is the main route of the NMDA-induced $^{45}Ca^{2+}$ release from the hippocampal neurons. Further experiments indicated that at least two distinct pools of $^{45}Ca^{2+}$ may be mobilized upon NMDA stimulation. One pool appears to be insensitive to intracellular calcium modulators. A possible role of calcium binding proteins in generation of $^{45}Ca^{2+}$ efflux from this pool will be discussed. The other one, which is sensitive to modulation by ryanodine, may be ascribed to a phenomenon of calcium-induced calcium release in ER. Our data suggest close functional relation between NMDA receptors, calcium binding proteins, and ER Ca^{2+} stores in postsynaptic sites of the rat hippocampal neurons, which may be critical for modulation of Ca^{2+}signal in NMDA receptors.

PHARMACOLOGICAL PROFILES OF NMDA-INDUCED EICOSANOID RELEASE IN RABBIT HIPPOCAMPUS IN VIVO

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Mechanisms of signal transduction in N-methyl-D-aspartate (NMDA) receptors encompass Ca^{2+} triggered, phospholipase A_2-mediated release of arachidonic acid (AA). Several eicosanoids, products of AA metabolism, are neuro- and vasoactive substances. The aim of the present in vivo study was to characterize pharmacologically interrelationship between the NMDA-induced influx of extracellular calcium to the rabbit hippocampal neurons and release of cyclo-oxygenase products: prostaglandin D_2, 6-keto-prostaglandin$F_{1\alpha}$ and thromboxane B_2. Changes in extracellular Ca^{2+} and in eicosanoid release were determined using microdialysis of the adult rabbit hippocampus, combined with detection of ^{45}Ca efflux and with RIA of the eicosanoids in dialysates. A 20-min pulse application of NMDA via microdialysis probe resulted in a prolonged, highly significant stimulation of the eicosanoid release, which was completely abolished by 10 μM indomethacin, a cyclo-oxygenase antagonist. Inhibition of thromboxane synthase by 0.1 mM furegrelate decreased by 75% thromboxane B_2 release with concomitant 100% increase in 6-keto-prostaglandin $F_{1\alpha}$ production. A dose-effect analysis disclosed that NMDA more potently stimulates eicosanoid release than induces a decrease in extracellular Ca^{2+} concentration. More surprisingly, 10 μM MK-801, a noncompetitive antagonist of NMDA receptors which completely abolished NMDA-induced decrease in extracellular Ca^{2+} concentration inhibited eicosanoid release only by 50%. These data suggest that coupling of NMDA receptors with AA releasing enzymes in neurons may be more complex than originally expected.

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VCAM-1 and clathrin heavy chain as possible metrazol induced genes in the rat brain.

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Differential RNA display is a novel, PCR-based technique of identification of differentially expressed genes. We employed this technique to compare mRNA species present in hippocampi of control rats and rats 1, 6, 24h after metrazol (50mg/kg, i.p.) induced seizures. Total RNA was reverse transcribed with an oligo-dT primer, modified by addition of two other bases at the 3' end. The first cDNA strand was amplified by PCR with the same primer and an arbitrary 10-mer, as the other primer, in the presence of ^{32}P-dCTP. Products of amplification were separated on a sequencing gel or Metaphor agarose. After autoradiography, fragments of the gel containing bands, intensities of which were increased by metrazol treatment, were cut out, and the DNAs were eluted and re-amplified with the same set of primers. The products of re-amplification were cloned into plasmid vector, sequenced by the dideoxy method, and their sequences were used for homology searches in data banks, via the EMBL sequence server. Out of 10 sequences, 2 were homologous to known rat genes: VCAM-1 and clathrin heavy chain. At present, we are performing northern and in situ hybridisations with obtained cDNA fragments, to confirm differential expression of their genes.

MAP-2 (MICROTUBULE-ASSOCIATED PROTEIN-2) PATTERN IN THE BARREL CORTEX IN MICE AFTER AN UNILATERAL PARTIAL LESION OF VIBRISSAL FOLLICLES.

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MAP-2 is a cytoskeletal protein of that occurs specifically in dendrites and thus is widely used as a dendritic marker. The barrel field is an area of the somatosensory cortex in rodents where granular cells form unique groups in layer IV (barrels) which show one-to-one correspondence to individual whiskers on the contralateral face. Neonatal lesions of vibrissae result in profound changes of cytoarchitectonics of the barrels. The distribution of dendrites has been examined in the barrel field after unilateral ablation of vibrissae sparing row C. Mice were lesioned on the day of birth and sacrificed after 8 weeks. Tangential brain sections through the layer IV were subjected to immunohistochemistry with a monoclonal antibody to MAP-2. A pattern of dendrites in the control hemisphere where all the barrel rows remain intact was examined. The density of stained cross-sections of single dendrites and clusters of dendrites, in terms of number per area, was higher in the barrel wall as compared to the barrel hollow and dendrites showed a more pronounced tendency to form clusters in the wall than in the hollow. In the contralateral hemisphere all but C row barrels were lost and the remaining barrels expanded to the surrounding area of the barrel cortex. To estimate the effect of the unilateral partial lesion of vibrissal follicles upon the dendrites pattern in the enlarged C row as well as in the denervated area, the precise measurements using a computer analysing system were done and compared to the control values.

THE ROLE OF PROTEIN KINASE C IN HISTAMINE-EVOKED STIMULATION OF cAMP PRODUCTION IN CHICK CENTRAL NERVOUS SYSTEM

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Recently, we have shown that histamine (HA) is a potent stimulator of cyclic AMP (cAMP) formation in the chick pineal gland and cerebral cortex. Detailed pharmacological characterization of this HA effect (at least in the pineal gland) suggests an involvement of a bizarre HA receptor, likely a novel (non-H_1, non-H_2, non-H_3) HA receptor subtype. To further study the nature of the HA-evoked stimulation of cAMP formation in the chick CNS, we were interested whether the HA action is a subject of any modulation by protein kinase C (PKC). This idea is reminiscent of a highly synergistic interaction between β-adrenergic (cAMP pathway) and α_1-adrenergic receptors (PLC/PKC pathway) in rat pineal. The study was carried out with a selective activator (phorbol 12,13-dibutyrate; PDB) and inhibitor (compound H-7; H-7) of PKC, and the compounds' effects on HA-stimulated cAMP formation were investigated on isolated pineal glands and cerebral cortical slices of 2-week old chicks, prelabeled with [^{3}H]adenine. HA (0.1μM - 1mM) concentration-dependently stimulated production of cAMP in both tested tissues; yet, the maximal effect of the amine was much greater in the pineal gland than in brain slices (approximately 1000% vs. 350-500% of control value at 100μM HA, respectively). PDB (0.1-100μM) given to preincubation, was inactive by itself, but it significantly increased the HA response, the effect being clearly stronger in the pineal gland. *Brain slices:* HA 0.5, 1 & 10μM - 173, 240 & 459 % control; HA + 1μM PDB - 257, 344 & 594 % control; *Pineal gland:* HA 0.5, 1 & 10μM - 190, 277 & 647; HA + 1μM PDB - 300, 393 & 1002 % control. In parallel study, a biologically inactive phorbol compound, i.e. 4β-phorbol, at concentrations up to 10μM did not modify the HA (1 & 10μM) effect. When combined with HA, H-7 (5-100μM) showed practically no significant action, suggesting that HA does not work via PKC. **Conclusions:** 1. Activation of PKC enhances the HA-evoked cAMP formation in the chick pineal and brain; 2. HA probably does not lead to stimulation of PKC in the chick CNS; 3. It is suggested that an unknown endogenous factor, capable of stimulating PKC, interacts with HA to produce an enhanced cAMP response.

Poster sessions - Peptides

BACLOFEN AND CENTRAL ACTIVITY OF ANGIOTENSIN II(3-8) AND (3-7) IN ALCOHOLIZED RATS

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In previous studies we obtain beneficial influence of fragment angiotensin II: 3-8 and 3-7 on learning and memory processes in chronically alcoholized rats. Chronic administration of ethanol reduces the function of central GABA-A and GABA-B receptors. The aim of this study was to investigate possible interaction between baclofen (agonist of the GABA-B receptor) and behavioral activity of AII 3-8 and AII 3-7 in rats chronically treated with ethanol. Ethanol used according to the method described by Wajda did not influence locomotor activity in the open field. Groups of rats which received baclofen or AII 3-8, AII 3-7 had the activity at the same level, but baclofen given with AII 3-8 and with AII 3-7 had this activity reduced in alcoholized group of rats. Alcohol-induced impairment of retrieval of passive avoidance in rats may eliminated by a single administration of AII 3-8 or AII 3-7. Baclofen did not effect the retrieval, but given together with AII 3-8 or with AII 3-7 improved the retrieval at the same level like the AII 3-8 and the AII 3-7. Alcoholized rats showed significant impairment of active avoidance acquistion. AII 3-8, AII 3-7, baclofen and coadministration of these peptides with baclofen improved acquisition of conditioned avoidance response (CAR) in both groups of rats.
Summary: We may suggest interaction between central activity of fragments angiotensin II 3-8 and 3-7 and baclofen, but explanation of this interaction needs further study.

Neuropeptide FF, an endogenous morphine antagonist, in the porcine and bovine central nervous system

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Neuropeptide FF (NPFF) was originally isolated from bovine spinal cord(*). In the rat it displayed strong antiopioid activity when administered intraventricularly(*) . NPFF was biochemically detected in the central nervous system of the rat, mouse, guinea pig, pig and man. In the rat, immunohistochemical studies revealed presence of NPFF-positive nerve fibers in the dorsal horn of the spinal cord, in the *medulla oblongata*, periaqueductal gray matter, hypothalamus, thalamus and septum, while NPFF-positive neurons were detected in the hypothalamus(**). In the porcine brain numerous NPFF-positive nerve fibers were detected in the *medulla oblongata*, particularly in the solitary nucleus and in the ventrolateral reticular nucleus. Scarce NPFF-positive nerve fibers were found in the periaqueductal gray matter, thalamus and hypothalamus, while only single NPFF-positive nerve fibers were found in the nuclei of the septum. In the bovine brain very numerous NPFF-positive nerve fibers were found in the *medulla oblongata* in the solitary nucleus and lateral reticular nucleus, as well as fibers "connecting" these two nuclei were visible. Single NPFF-positive nerve fibers were found in the periventricular hypothalamus, infundibulum and nuclei of thalamus. No NPFF-positive nerve fibers were found in the septum. Analysis of the distribution of immunoreactivity to NPFF in the rat, pig and cow suggests signifficant degree of conservation. Except for the differences in the number of NPFF-positive fibers, the overall pattern of distribution seems to be similar in mammalian species as distant as rat, pig and cow.

(*)Yang et al.(1985), PNAS 82, pp.7757-7761
(**)Lee et al.(1993), Eur. J. Neurosci. 5, pp.1339-1348

ENDOGENOUS NEUROPEPTIDES ARE INVOLVED IN LONG-TERM POTENTIATION AND DEPRESSION IN RAT BRAIN OLFACTORY CORTEX SLICES

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The purpose of this study was to test the hypothesis that during tetanization some substances, which are capable to induce and to maintain of long-term potentiation/depression, could release. The experiments were carried out on the rat brain olfactory cortex slices. Perfusates were collected from the donor slice for 20 min during tetanization of the lateral olfactory tract (four times: 100Hz, 2-4V for 30s, with 5-min interval). In recipient slices the collected perfusates induced the reactions which were contrary to those developed after tetanization in the donor slices. Perfusates collected from donor slices were separated by ultrafiltration into two fractions according to molecular weights < 50 and > 50 kDa. The latter induced depressive reactions in recipient slices. Fractions < 50 kDa produced three kinds reactions in recipient slices: potentiation, depression and their absence. To establish chemical nature of releasing factors fractions > 50 kDa was treated by immobilized enzyme trypsine. After treatment this perfusate was not able to induce the initial reaction as before treatment. Data obtained evidence that tetanization of donor slice result in a release neurochemical factors likely polypeptides which are capable to induce potentiation/depression in recipient slices.

Neurochemical coding of pelvic neurons projecting to some porcine male genital organs.

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The anterior pelvic ganglion (APG) of the boar is a bilateral structure located in the angle between the cranial end of the seminal vesicle and vas deferens. Combined retrograde tracing and double-labelling immunofluorescence were used to investigate the co-existence of putative transmitters in APG neurons that project to the ductus deferens and seminal vesicle in sexually immature boars (n=8). Fluorescent tracer Fast Blue (FB) was injected into the wall of the left vas deferens in 4, or into the seminal vesicle in remaining 4 boars during laparotomy. After 3-week survival period animals were deeply anaesthetized, perfused with buffered paraformaldehyde and APGs were collected. Serial sections of the ganglia were processed for double-labelling immunofluorescence. The APG was found to contain many FB-positive (FB^+) neurons projecting to the vas deferens or seminal vesicle. Immunohistochemical characteristics of both subpopulations of FB^+ neurons were very similar. The vast majority of FB^+ neurons (90%) showed tyrosine hydroxylase-immunoreactivity (TH-IR). Only small numbers of FB^+ neurons (2-6%) displayed vasoactive intestinal peptide (VIP)-, choline acetyltransferase (ChAT)-, or nitric oxide synthase (NOS)-IR; virtually all these neurons were TH-immunonegative. Many FB^+ neurons (25%) contained neuropeptide Y (NPY)-IR; the majority of them (60%) were also TH-IR. Many FB^+ neurons (30%) were Met^5-enkephalin-Arg^6-Gly^7-Leu^8 (MEAGL)-IR; the vast majority of them (90%) contained also TH-IR. Solitary FB^+ neurons (0.5%) were calcitonin gene-related peptide (CGRP)-IR; virtually all these neurons were TH- or substance P (SP)-negative. Many FB^+ neurons (10%) contained galanin (GAL)-IR; The majority of them (65%) were also TH-IR. The analysis of adjacent sections revealed that some of FB^+ neurons contained more than one neuropeptide. Following combinations of neuropeptides and/or TH existing in FB^+ neurons were found: NPY/VIP, NPY/MEAGL/TH, NPY/CGRP, ChAT/VIP, ChAT/NOS. FB^+ neurons received very strong CGRP-, ChAT- and substance P (SP)-IR innervation.

CHOLECYSTOKININ MODULATION OF ^{3}H-FLUNITRAZEPAM BINDING IN RAT CEREBRAL CORTEX

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The finding that BD agonists as e.g. flunitrazepam antagonize cholecystokinin (CCK)-induced activation of rat hippocampal neurons suggests a modulation of GABA/BD receptor complex by CCK. Specific ^{3}H-flunitrazepam (2.0 nM) binding was not changed by CCK-8 (10^{-9} - 10^{-5} M) but increased (by 129.1 %) by CCK-4 (10^{-9} M) when binding was performed in the presence of 0.1 % bacitracin and 0.1 mM PMSF at 37° C but not at 0 - 4° C. Both CCK peptides did not significantly modify GABA-stimulated (10^{-8} - 10^{-6} M) ^{3}H-flunitrazepam (2.0 nM) binding in the absence of protease inhibitors in either 37°C or 0 - 4°C. However, in the presence of 0.1 % bacitracin and 0.1mM PMSF at 37° C CCK-8 (10^{-6} M) decreased GABA (10^{-6} M)-stimulated ^{3}H-flunitrazepam binding (159.1 ± 0.7 %) almost to the level of unstimulated control ^{3}H-flunitrazepam binding (112.6 ± 12.1 %, $p < 0.05$). The present reults suggests CCK modulation of BD receptors in rat cerebral cortex which probably reflects the functional antagonism between the CCKergic and GABA/BD systems in vivo.

THYROLIBERIN (TRH) AND RELEASE OF VASOPRESSIN AND OXYTOCIN FROM THE RAT HYPOTHALAMO--NEUROHYPOPHYSIAL EXPLANTS *in vitro*

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Thyroliberin (Thyrotropin-releasing hormone: TRH) is known to be distributed throughout the central nervous system and to act as a neurotransmitter or neuromodulator. Recently, TRH was shown, in own studies, to modify the vasopressin and oxytocin release in euhydrated, dehydrated, salt-loaded as well as haemorrhaged rats.

This report deals with the effect of TRH on the basal and potassium--evoked release of vasopressin and oxytocin from the rat hypothalamo--neurohypophysial explants *in vitro*. Vasopressin and oxytocin were estimated by RIA.

Incubation of the hypothalamo-neurohypophysial explants in Locke's solution containing 28 nmol/l TRH resulted in an inhibition of the vasopressin as well as oxytocin release under basal conditions and statistically significant during depolarization due to excess (56 nmol) potassium.

These data suggest the involvement of TRH in the regulatory mechanisms of vasopressin and oxytocin release; the inhibitory effect of TRH cannot be excluded.

Prostaglandins Mediate the Pituitary-Adrenocortical Stimulation by Adrenergic Agonists

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Catecholamines present in high concentrations in the hypothalamus are generaly found to stimulate the hypothalamic-pituitary-adrenal (HPA) axis via adrenergic α_1- and α_2-receptors in the parvocellular region of the paraventricular nucleus. Prostaglandins (PGs) may stimulate the release of ACTH from the anterior pituitary corticotrops either directly or by hypothalamic release of CRH. Noradrenaline stimulates both PGE_2 release and ACTH secretion. A possible mediation of central PGs in the stimulation of the HPA axis by adrenergic agonists was investigated in unanesthetized rats. Adrenergic α_1-, α_2- and β-receptor agonists, phenylephrine (30 μg), clonidine (10 μg), and isoproterenol (20 μg) and noradrenaline (10 μg) and adrenaline (10 μg) given icv increased the serum corticosterone levels 1 h later. Pretreatment with cyclooxygenase and PGs synthesis inhibitor indomethacin (2 mg/kg ip or 10 μg icv) abolished the corticosterone response to phenylephrine, and considerably diminished the response to noradrenaline. Also the clonidine-elicited increase in serum corticosterone level was reduced by pretreatment with indomethacin. However, indomethacin did not markedly diminish the rise in corticosterone levels induced by isoproterenol and adrenaline.

These results indicate that PGs are considerably involved in the stimulation of HPA axis by phenylephrine, clonidine and noradrenaline, which act predominantly on central α_1- or postsynaptic α_2-adrenergic receptors. Prostaglandins are not significantly involved in the stimulation of HPA axis by isoproterenol or adrenaline which stimulate mainly central β-adrenergic receptors.

The Significance of Central Adrenergic System in the Met-Enkephalin-Induced Corticosterone Secretion

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Opiates affect the activity of the hypothalamic-pituitary-adrenal (HPA) axis via opiatergic neurons and specific opioid receptors in the hypothalamus. Enkephalins coexist with corticotropin releasing hormone in the paraventricular nucleus. Presynaptic opioid heteroceptors may modulate the release of catecholamine neurotransmitters which activate the HPA axis. The involvement of central opioid and adrenergic mechanisms in the HPA response, measured through corticosterone secretion, to central stimulation with metenkephalin was investigated in unanesthetized rats.

In conscious rats met-enkephalin (100 μg) and its stable analogue met-enkephalinamide (2 μg) (DADM), a δ-opioid receptor agonists, administered intracerebroventricularly increased dose-dependently the serum corticosterone levels. Pretreatment with naloxone, an opioid receptor antagonist totally antagonized the corticosterone response to these opioid agonists. Prazosin, (0.1 μg icv) an α_1-adrenergic antagonist, and yohimbine, (0.01 μg) an α_2-adrenergic antagonist, considerably reduced the increase in serum corticosterone levels induced by met-enkephalin. Pretreatment with propranolol, (10 μg) a β-adrenergic antagonist, did not significantly affect the rise in serum corticosterone levels elicited by met-enkephalin. Lesions of central noradrenergic neurons by a selective noradrenergic neurotoxin DSP-4, 50 mg/kg ip 8 days prior to DADM, considerably reduced the hypothalamic noradrenergic content and markedly diminished the DADM-induced increase in the serum corticosterone level.

These results indicate that in the stimulating action of enkephalin on HPA axis opioid receptors interact with noradrenergic neurons and adrenergic α-receptors whereas β-receptors do not play any significant role in this stimulation.

THE EFFECT OF MELATONIN ON CHRONIC MILD STRESS-INDUCED DISTURBANCES OF THE CIRCADIAN RHYTHM IN THE LOCOMOTOR ACTIVITY IN RATS

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Melatonin (MEL), a neurohormone of the pineal gland, seems to be a physiological mediator of the light-dark cycle information in many species. After a phase shift of the LD cycle, circadian activity rhythms are entrained by MEL in humans and laboratory animals. Previously, we have shown that the chronic mild stress procedure (CMS) disturbed the circadian rhythm of the locomotor activity in rats. At present, we decided to determine whether repeated treatment with MEL would prevent those CMS-induced rhythm disturbances. The experiment was carried out on male Wistar rats 3 month old housed individually with free access to food and water on a light-dark schedule (LD 12:12). For four weeks a half of the rats was subjected to CMS and a second half was not stressed. The rats received MEL (1 mg/kg) or vehicle, once daily. In fifth week, all four groups of rats were kept in the constant light for 24 h a day (LL cycle) and their locomotor activity was continously recorded using Animex actometers and an IBM computer system. In the LL cycle, a free running rhythm appeared in the locomotor activity of all rats. CMS decreased the amplitude of the activity rhythm and shifted its phase in comparison to the control. MEL itself did not affect the rhythm parameters in the unstressed rats but considerably prevented the disturbances in the stressed rats. The results suggest a beneficial effect of MEL on the circadian rhythmicity during chronic stress.

THE EFFECTS OF MULTIPLE ADMINISTRATIONS OF NEUROLEPTICS ON NEUROPEPTIDE Y CONTENT IN THE RAT HYPOTHALAMUS

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In the present study we have investigated the effects of 14 or 28-day intraperitoneally administration of Chlorpromazine (CPZ) (2 or 10 mg/kg); Haloperidol (HAL) (0.5 or 2 mg/kg); Sulpiride (SULP) (50 or 100 mg/kg); Clozapine (CLOZ) (10 or 25 mg/kg) on NPY concentration (NPYLI) in the hypothalamus. NPYLI was measured by radioimmunoassay. After 14 as well as 28 days of treatment only the classical neuroleptics - HAL or CPZ remarkably elevated NPYLI. CLOZ in the higher dose slightly increased hypothalamic NPY content after chronic administration. SULP had no effect. Single injection of the D_2 agonist Quinpirole (3 mg/kg ip) had no effect by itsefl but completely reversed changes induced by CPZ or HAL. Futhermore, we claimed that 14-day treatment with D_1 antagonist SCH23390 (1 mg/kg ip) failed to reveal any effect on hypothalamic NPY. However, co-administration this compound with SULP evidently increased NPYLI. In conclusion we suggested that NPY may contribute in the mechanism of action of neuroleptics. The increase of hypothalamic NPY may be involved in endocrinologic disturbances described during treatment with typical neuroleptics. Our data indicate that this effect is elicited by D_1 and D_2 receptor blockade.

THE EFFECT OF MELATONIN ON THE OXYTOCIN RELEASE FROM THE HAMSTER NEUROINTERMEDIATE LOBE *in vitro*.

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Melatonin (Mel) was found to modify oxytocin (OT) release from the rat hypothalamus (Yasin et al., Endocrinology 1993, 132:1329-1336) and neurointermediate lobe (Juszczak et al., J. Pineal Res. 1992, 12:1-6) *in vitro*. The purpose of the present study was to investigate whether Mel affects *in vitro* release of OT from the male Syrian hamster neurointermediate lobe and whether such effect is dose-dependent.

The effect of various concentrations of Mel (10^{-7} M/l, 10^{-9} M/l and 10^{-11} M/l) on basal and K^+-evoked OT release from the neurointermediate lobe *in vitro* was determined. The content of OT in the incubation medium (Krebs-Ringer bicarbonate solution) was measured by radioimmunoassay.

Under basal conditions, MT inhibited OT release from the neurointermediate lobe in all concentrations studied. 10-fold excess of potassium caused a significant increase of OT release. However, the rise of OT release stimulated by excess potassium, was significantly lower when 10^{-9} M/l Mel was used; after administration od 10^{-7} M/l or 10^{-11} M/l Mel, the K^+-evoked release of OT was not different from the control.

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Prostaglandins Inhibit the Vasopressin-Induced Corticosterone Response

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Corticotropin-releasing hormone (CRH) and vasopressin (VP) are the major neuropeptide mediators in the secretion of adrenocorticotropic hormone (ACTH). Vasopressin is considered to potentiate the stimulatory effect of CRH on the anterior pituitary. The effect of VP on the pituitary-adrenocortical activity, measured through corticosterone secretion, and a possible involvement of prostaglandins (PGs) in this stimulation was investigated in conscious rats.
VP administered icv (5 μg/rat) or ip (5 μg/kg) elicited a dose related increase in the serum corticosterone levels 1 h later. The maximum corticosterone response did not significantly differ after both routes of VP administration. On a molar basis, VP given ip was more potent in inducing corticosterone response than when administered icv, indicating that VP receptors on anterior pituitary are best accessible by systemic VP administration. Indomethacin, a cyclooxygenase and PGs synthesis inhibitor, given ip (2 mg/kg) or icv (1-10 μg) 15 min prior to VP considerably reduced the corticosterone response to both icv or ip administered VP.

These results indicate that VP itself can release ACTH from pituitary corticotropes in vivo and PGs significantly mediate the stimulatory effect of VP at both the hypothalamus and pituitary levels.

INVOLVEMENT OF THE NEUROPEPTIDES CGRP AND SP IN NEUROGENIC BLOOD FLOW RESPONSES OF THE DURA MATER IN THE RAT

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An involvement of the vasoactive peptides CGRP and SP in neurogenic inflammation has been shown for various tissues. The aim of this study was to examine the effect of these sensory neuropeptides on the meningeal perfusion. A preparation was developed in which the dural blood flow in branches of the medial meningeal artery was monitored with a laser Doppler flowmeter in barbiturate anesthetized rats. Stimulating electrodes were located near the superior sagittal sinus to stimulate dural afferents. Electrical stimulation (pulses of 0.5 ms, 10-20 V, 5-10 Hz) for 30 s caused a repeatable and consistent increase of the blood flow for 1-2 min. These effects could be dose-dependently inhibited by topical application of the CGRP antagonist h-αCGRP$_{8-37}$ but not by the NK_1 antagonist RP 67850. Accordingly, administration of h-α CGRP at a concentration of 10^{-4} M increased the basal blood flow, whereas substance P and the more stable substance P analog septide at this concentration did not. We conclude that stimulation of trigeminal afferents innervating the dura mater releases neuropeptides from peptidergic afferent terminals which lead to vasodilatation and increase of the meningeal blood flow. This response seems to be mainly caused by CGRP that may directly act on the vascular smooth muscle, whereas substance P may require the endothelium of arterial vessels to mediate vasodilatation, probably by the release of NO. The increase of meningeal blood flow may be an important element of neurogenic inflammation in the meninges.

INFLUENCE OF CHOLECYSTOKININ (CCK-8) AND YOHIMBINE ON SUPRASPINAL MODULATION OF NOCICEPTIVE PROCESS.

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The aim of our investigations was to evaluate the role of (CCK-8) and noradrenergic system and their interaction in brainstem reticular formation on nociceptive process. The experiments were carried out on conscious rabbits with permanently implanted electrodes into motor-sensory cortex (MSC), ventro-lateral posterior thalamic nuclei (NVPL), hippocampus (HIP), and lateral reticular formation (LRF), where additionally a cannula was implanted. CCK-8 in doses of 100ng and 200ng/rabbit and Yohimbine (in concentr. 7mg/ml) were administered locally. Nociceptive stimulation (NS) was performed by means of electrical pulses (60Hz, 8mA, 5s) applied to the front paw. Bioelectrical activity (BA) before and after NS was analyzed by means of spectral analysis (FFT), Autoregressive Model (AR) and directed transfer function (DTF). NS changed power spectra of BA in analyzed structures and increased the synchronism between NVPL and MSC, HIP and MSC and between HIP and NVPL. NS after CCK-8 in a dose of 100ng had no significant influence either on BA or the synchronism between structures. NS after the dose of 200ng produced significant changes in BA of MSC and NVPL and increased synchronisation between NVPL and MSC, and between HIP and MSC. NS after Yohimbine changed power spectra of BA in MSC and NVPL, and increased the synchronism between NVPL and MSC, NVPL and HIP, and between LRF and MSC. We also observed decreased synchronism between HIP and LRF. NS after administration of Yohimbine together with CCK-8 (only in the 100ng dose) changed BA in MSC. The analysis of DTF revealed increased synchronism between the brain structures: NVPL and MSC, HIP and MSC (in both doses of CCK-8). Additionally, we observed increased synchronism between LRF and NVPL after the 100ng dose of CCK-8 and between LRF and MSC after the dose of 200ng CCK-8.
These results suggest that Yohimbine administered together with CCK-8 (in both doses) to LRF have inhibitory effect on nociceptive process.

IMMUNOHISTOCHEMICAL STUDIES ON THE CATECHOLAMINERGIC REGULATION OF NEUROPEPTIDE Y NEURONS IN THE RAT CEREBRAL CORTEX

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Previous studies indicated dopaminergic regulation of the neuropeptide Y (NPY) expression in neurons of some brain structures. The present study examineed the possibility of similar catecholaminergic regulation in the cerebral cortex. Rats were treated with compounds acting on catecholamine receptors; after 24 h, NPY-immunoreactive (-ir) neurons were counted in the cortex and the mean density of stained neurons per microscopic field was assessed.

It was found that the blockade of dopaminergic receptors by haloperidol or the specific D1 receptor blockade by SCH23390 caused a significant increase in the density of NPY-ir neurons. The latter compound affected mainly inner cortical layers. Stimulation of α_2 receptors by clonidine slightly increased the density of NPY-ir neurons, mainly in outer cortical layers. Neither the specific D2 receptor blockade by sulpiride, nor the α_1- or β-adrenergic receptors blockade by phenoxybenzamine or propranolol, respectively, induced any significant changes in the cortical NPY-ir.

The obtained results suggest an inhibitory dopaminergic control of the NPY content - mainly via D1 receptors - and a slight stimulatory α_2 regulation in neurons of the rat brain cortex.

Macrophages express mRNAs for nerve growth factor and for convertases furin and PC1 in the lesioned sciatic nerve.

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Distal segment of the disconnected peripheral nerve releases the nerve growth factor (NGF). This factor stimulates the neuritic outgrowth and the proliferation of myelin-producing Schwann cells. Identification of cells which produce NGF is important for understanding the mechanism of peripheral nerve regeneration. As deduced from data on spatial and temporal distribution of NGF-like activity released transiently from disconnected distal stump [Richardson and Ebendal, Brain Res. 246:57,1982], such cells should be present both within endoneurial and perineurial tissues. So far cells with these characteristics were not shown *in situ*. Most knowledge on NGF comes from culturing the denervated cells. In these conditions NGF has been shown to be produced by Schwann cells. Our data do not support this notion since we show by *in situ* hybridization that after lesion, in the perineurium and endoneurium, the macrophages, but not Schwann cells transiently express NGF. Spatial and temporal patterns of NGF mRNA expression have been superimposable, both anatomically and cellularly, with the sites of myeloperoxidase activity, a macrophages marker. Similar hybridization patterns have also been observed with mRNA for two putative convertases furin and PC1, both thought to be involved in the processing of inactive NGF precursor into the active factor. We suggest that NGF secretion involves an infiltration by hematogenous cells which are able to synthesize and process its precursor. Our findings indicate a functional co-operation between the immune system and the nervous system during nerve repair.

OPPOSING GASTRIC MOTOR RESPONSES TO VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) AND PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) ON THEIR MICROINJECTION INTO NUCLEUS RAPHE OBSCURUS (nRO) OF THE RAT. Z.K. Krowicki[1], A. Arimura[2], and P.J. Hornby[1]; [1]Department of Pharmacology, LSU Medical Center, New Orleans, LA, USA; [2] U.S.-Japan Biomedical Research Laboratories, Tulane University Hebert Center, Belle Chasse, LA, USA.

Little is known about the functional role of putative neurotransmitters in the nRO in the control of gastric motor function, although VIP and PACAP have been detected in the cell bodies and/or fibers and terminals in this brainstem nucleus. Both these peptides show high sequence homology. Therefore, we investigated the effect of microinjection of VIP and PACAP38 (1, 10, and 100 pmol; 30 nl), as well as saline (30 nl), into the caudal nRO of α-chloralose-anesthetized rats on intragastric pressure (IGP), pyloric and greater curvature motility, heart rate, and blood pressure. Pontamine sky blue (30 nl) was used for histological confirmation of the microinjection sites. Peak changes in IGP, areas of the response (ARIGP) below (-) or above (+) baseline levels, and changes in pyloric and greater curvature motility indexes (PMMI and GCMMI, respectively) are shown as means ± SE.

	VIP (100 pmol; n = 6)	PACAP38 (100 pmol; n =5)
Peak IGP (cm H_2O)	-2.54 ± 0.16*	+2.40 ± 0.51*
ARIGP (cm^2)	-4.89 ± 1.04*	+0.54 ± 0.12*
PMMI	-4.28 ± 1.38*	+3.70 ± 3.08
GCMMI	-1.16 ± 0.38*	+1.10 ± 0.5

* P<0.05 vs. corresponding saline by ANOVA with subsequent Newman-Keuls test

These data indicate that activation of VIP receptors in the nRO inhibits gastric motor function, whereas activation of PACAP receptors in the same site has the opposite effect, namely stimulation of IGP. Thus, in the nRO, the effects of these two closely-related peptides on gastric motor function are mediated through different receptors. Supported by PHS grant DK42714.

Studies on in vitro degradation of JP-analogue of C-terminal fragment of substance P-in rat brain; regulatory aspects.

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One can find just a few papers concerning the possible wyas of regulation of the hydrolysis of neuropeptides with peptidases (proteases) in brain. We elaborated the simple method of assay of enzymes-acting on the JP i.e. hexapeptide $Glp^6[^{125}I\text{-}Tyr^8]SP_{6-11}$ - based on the separation of undegraded JP and different products of its hydrolysis - withut HPLC - due to difference of their electric charge. We found that inhibiting of one enzyme (e.g.Z) causes the increase of the other peptidase (e.g.X) acting-at the same time-on the (other peptide bond of) JP. We draw the hypothesis that the products of (action on JP) one peptidase are the strong inhibitors of the other peptidase. The stimulation of phosphorylation of proteins of synaptosomes of hipocampus with both kinase C and CaM triggers inhibition of peptidase X and stimulation of peptidase Z. In neclear fraction of cortex (?!) activation of kinase C causes the rise of Z SH-dependent) with parallell drop of X(metalloenzyme); activation of CaM kinase makes the opposite effects. Besides, different peptides (including LH-RN and dynorphin 1-13) might also trigger described phenomena. Capsaicin (the SP depleting agent) might cause rise of peptidase Z and inhibition of X (possibly "enkephalinase").

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Poster sessions - Dopaminergic system

INHIBITION OF CORTICOSTERONE SYNTHESIS BY METYRAPONE DECREASES DOPAMINE D1 RECEPTORS IN RAT BRAIN

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The growing body of experimental and clinical data are showing an interaction between glucocorticoids and brain dopaminergic (DA) systems. However, information on the effect of these hormones on brain DA receptors is still limited and questionable. Therefore the aim of this study was to investigate the effect of the corticosterone synthesis inhibitor metyrapone on the density of DA D1 receptors in the brain areas that show high level of both DA D1 and glucocorticoid receptors. The method of autoradiography (D1 antagonist [^{3}H]SCH 23390) was used to mesure regional receptor changes. Modulation of corticosterone secretion (metyrapone; two injections of 150 and 50 mg/kg i.p., given 20 and 3 h before sacrifice, respectively) induced decrease in the D1 receptor specific binding over the basal ganglia. In the striatum, the decrease in [^{3}H]SCH 23390 binding was stronger in the medial (31-39%) than in the lateral (24-27%) part. Similar decreases as those in the striatum were observed in the olfactory tubercle (32%) and nucleus accumbens shell (29%), whereas in the nucleus accumbens core only a slight 13%, decrease in the D1 dopamine receptor level was observed. The obtained results show that brain dopamine D1 receptors may be regulated by alterations in the corticosterone level; hence they suggest that adrenal hormones may modulate the information flow via dopamine D1 receptors.

POLYAMINES ATTENUATE THE CATALEPSY ELICITED BY HALOPERIDOL

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The N-methyl-D-aspartate (NMDA) receptor is believed to be implicated in psychiatric disorders. Polyamines (PA): spermidine (SPD) and spermine (SPM), but not putrescine (PUT) bind to a specific polyamine site on this receptor molecule. Furthermore, PA have modulatory effects on dopaminergic systems (Genedani, 1994) and interact with $GABA_A$ receptor (Doyle, 1992). Ifenprodil, the polyamine-sensitive sites antagonist may label sigma receptors in rat brain, too (Hashimoto, 1994). Haloperidol (HAL) beside the more selective blockade of D_2 than D_1 receptor has a weak affinity for 5-HT_2 and alpha$_1$-receptors. In addition is not devoid of potential sigma antagonist activity.

We examined the effect of SPD and SPM on the behavioural response to HAL in rats. Catalepsy and the locomotor activity was measured in animals after both acute and subchronic administration of HAL in combination with a single dose of PA. To assess the interaction with HAL a combination of SPD (40 mg/kg) i.p. + HAL (1.0 mg/kg) i.p. for 1 or 14 days was administered. This resulted in a significant attenuation of catalepsy evoked by single or multiple HAL injections. Accordingly, an acute and subchronic treatment with HAL joint with SPM (20 mg/kg) i.p. markedly alleviated the catalepsy elicited by HAL. Increased locomotor activity in rats treated subchronically with HAL was potently diminished only by SPM.

There findings indicate that PA may alter the behavioural response to HAL.

TRIHYDROXY-N-PROPYLNORAPORPHINE AFFECTS THE PATTERN OF ONGOING DISCHARGE IN SINGLE RENAL SYMPATHETIC NEURONS

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Changes in spontaneous sympathetic discharge observed after sulpiride injection suggest that dopaminergic mechanisms influence the background firing of postganglionic sympathetic neurons. To obtain deeper insight into this question we assessed the effect of trihydroxy-N-propylnoraporphine (TNPA), dopamine D_2 receptor agonist, on the pattern of spontaneous sympathetic discharge. In anaesthetized rabbits single fibre recordings were obtained from the central stump of the renal nerve. Spontaneous activity was used to compile interspike interval histograms and to measure the discharge rate. In control conditions the mean frequency of discharge was 1.78 ± 0.2 spikes/s (x ± S.D.; n = 6). The preferred and longest interspike intervals amounted to 20 ms and 1747 ± 42 ms. TNPA in a dose of 0.02 mg/kg, i.v., significantly lowered the rate of discharge (to 1.24 ± 0.02 spikes/s, i.e. to 69.7% of control; $P < 0.01$). The preferred and longest interspike intervals were lengthened to 30 ms and 2437 ± 313 ms (to 150% and 139,5% of control values). TNPA in a dose of 0.04 mg/kg decelerated the discharge rate to 1.22 ± 0.28 spikes/s (to 68.5%). The preferred and longest interspike intervals were increased to 116.5% and 164.4% of control. Opposite influence of sulpiride and TNPA on firing of single renal sympathetic neurons at least in fibres displaying similar discharge rate suggests that dopaminergic mechanisms exert tonic effect on the pattern of sympathetic postganglionic nerve discharge.

Influence of New Derivatives of 3-Aminooxazolidinone on Serotoninergic and Dopaminergic Neurotransmission in Rat's Brain

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AS-8 (5-morpholinemethyl-3-(4chlorobenzylideneamino)-2-oxazolidinone) is a potential psychotropic drug exhibiting antidepressant-like activity in several behavioral tests on rats. It has also been demonstrated that AS-8 was capable of stimulating 5-HT synthesis in mammalian brain. For the purpose of QSAR (Quantitative Structure-Activity Relationship) several structural analogues of AS-8 have been prepared. Some of the compounds have exhibited potency to influence the turnover of serotonin and/or dopamine in the rat's brain in a dose dependant manner. The metabolic tests have shown that the compounds exhibited structure-dependant biochemical activity connected to dopaminergic and serotoninergic neurotransmission.

IMPACT OF 5-HT_{1A} AGONISTS ON THE ALTERATIONS OF DOPAMINE OUTFLOW IN THE RAT PREFRONTAL CORTEX EVOKED BY SOCIAL INTERACTION OF RATS

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In the present study we (1) analyzed the impact of stress/fear induced by social interaction on alterations of dopamine (DA) outflow in the rat prefrontal cortex (PFC), and (2) tried to determine whether such alterations are influenced by the classic anxiolytic drug diazepam (DIA) and the novel, putative one ipsapirone (IPSA). Alterations in DA outflow were measured by transcortical brain microdialysis and HPLC measurements of DA concentrations. The stress/fear induced by social interaction was studied in an experimental paradigm in which intruders with dialyzing probes implanted into the PFC, were exposed to a dominant, aggressive opponent. Such an interaction at the behavioral level resulted in an aggressive interaction between the dominant and the intruder, and in consequence, subjected the intruders to a totally submissive behavior. At the level of dopaminergic neurotransmission in the rat PFC, such an interaction led to enhancement of DA outflow, measured as an increase in DA concentrations in the dialysate. IPSA given in doses of 10 and 5 mg/kg enhanced the outflow of dopamine in the rat PFC; a dose of 2.5 mg/kg was ineffective. That effect was antagonized by NAN-190 (1 mg/kg) and WAY 100135 (10 mg/kg) antagonists of 5-HT_{1A} receptor. These results indicate that the effects of IPSA on the outflow of DA, observed in the present study, involve serotonergic receptors of the 5-HT_{1A} subtypes. Diazepam (DIA) had a different profile of action on the outflow of DA in the rat PFC. DIA, given in a dose of 10 mg/kg, evoked attenuation of the outflow of DA in the rat PFC.

IPSA (10 and 2.5) failed to alter the enhancement of DA outflow induced by stress/fear of social interaction. Diazepam (10 mg/kg) abolished the effects of stress/fear on the outflow of DA.

It is concluded that stress/fear induced by social interaction i.e. exposure to the dominant aggressive opponent, enhances the outflow of DA in a subordinate animal. The alteration in the DA outflow evoked by social interaction are antagonized by the classic anxiolytic DIA, but not by the novel anxiolytic which acts via activation of serotonergic receptors of the 5-HT_{1A} subtype.

EFFECTS OF COMPROMISED ENERGY PRODUCTION ON DOPAMINERGIC NEURONS *IN VITRO* AND *IN VIVO*.

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Metabolic defects may be a causative factor in Parkinson's Disease. *In vitro*, the effect of compromised energy production on dopaminergic neurons was investigated in cultured mesencephalic cells exposed to malonate, an inhibitor of succinate dehydrogenase. Treatment with malonate (10-50 mM, 24 h) produced a dose-dependent decrease in ^{3}H-dopamine (DA) uptake but did not significantly affect ^{14}C-GABA uptake. *In vivo*, a unilateral infusion of malonate (0.25-1.0 μmol) into the left substantia nigra of the rat dose-dependently reduced the concentrations of DA in this region as well as in the striatum of the infused side compared to the contralateral regions when measured 7 days after infusion. Similarly, a unilateral infusion of malonate (0.5-4 μmol) into the striatum of rats or mice dose-dependently reduced striatal DA concentrations. GABA concentrations in these brain regions were also affected by malonate treatment. Preliminary data suggest that an intrastriatal infusion of malonate (4 μmol) produced a greater percent decrease in striatal DA (80%) than striatal GABA (59%) whereas the percent loss of these two transmitters within the substantia nigra was similar after an intranigral infusion. Nigrostriatal dopaminergic neurons are destroyed by the systemic administration of methamphetamine to mice. It is thought that such treatment produces a severe metabolic stress within the dopaminergic neurons which contributes to the neurodegeneration. We have found that an intrastriatal infusion of malonate potentiated the neurotoxic actions of systemically administered methamphetamine in mice (greater striatal DA loss). It is concluded that reduced energy metabolism and/or increased metabolic stress is detrimental to DA neurons, particularly the striatal DA terminals.

THE EFFECT OF CHRONIC COCAINE "BINGE" ADMINISTRATION ON [^{3}H]DOPAMINE UPTAKE IN THE NUCLEUS ACCUMBENS AND STRIATUM OF THE RAT

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It is currently assumed that the cocaine-induced inhibition of the dopamine uptake in the mesolimbic system underlies a reinforcing action of this drug; however, less is known how the dopamine uptake is adjusted following discontinuation of chronic cocaine administration. Therefore in the present study we investigated the effect of cocaine on the synaptosomal dopamine uptake in the nucleus accumbens and striatum of male Wistar rats chronically treated with cocaine ("binge" treatment, 15 mg/kg i.p., once every hour for 3 h, for 8 days). The rats were killed by decapitation 24 hours after the last cocaine injection, and the effect of cocaine (10^{-9} - 10^{-5}) on the [^{3}H]dopamine uptake was assesed. The obtained results confirmed that cocaine is a potent inhibitor of the dopamine uptake in both these structures. In the nucleus accumbens obtained from rats treated chronically with cocaine, the IC_{50} value of the drug was significantly lower than in control rats. On the other hand, in striatal synaptosomes, the IC_{50} value only tended to decrease. The above data suggest that chronic cocaine "binge" administration exerts more extensive influence on the dopamine uptake in the mesolimbic system than in the nigrostriatal one.

A COMPARISON OF BEHAVIORAL AND BIOCHEMICAL EFFECTS AFTER CHRONIC TREATMENT WITH HALOPERIDOL AND PIMOZIDE

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Chronic treatment with neuroleptics leads to development of tolerance followed by symptoms of dopaminergic supersensitivity upon withdrawal. We compared the behavioral and biochemical effects of withdrawal from a classic neuroleptic, haloperidol, and pimozide, a neuroleptic with calcium blocking properties in male Wistar rats. The biochemical investigations included: dopamine D1 receptors ([^{3}H]SCH-23390 binding sites in the limbic structures), calcium channel receptors ([^{3}H]nitrendipine binding sites in the cortex) and the level of dopamine (DA) and its metabolites (DOPAC, HVA) measured by HPLC in the striatum, nucleus accumbens and cortex. In behavioral studies we investigated motor and stereotypy responses to dopamine agonist - apomorphine. Neuroleptics, haloperidol, 1 mg\kg ip and pimozide, 4 mg/kg ip, were administered daily for 14 days and withdrawal effects were investigated 24 h and 9 or 12 days after the last dose. Apomorphine, 1 mg/kg was given immediately before the behavioral tests. The motor and stereotypy responses to apomorphine were significantly higher in groups treated with haloperidol (about 80% and 60% resp.) than in apomorphine control, both 24 h and 9 days after the last dose. In contrast, during withdrawal period from pimozide (24 h and 9 d), motor and stereotypy responses to apomorphine were strongly reduced (about 65% and 90% resp). The binding studies have shown, that haloperidol significantly increased both the density of calcium channels and dopamine D1 receptors (resp. 60% and 45%) in the brain structures during withdrawal. No changes in Kd were observed in any group. The treatment with pimozide did not affect the density and affinity of [^{3}H]SCH 23390 binding sites and the density of [^{3}H]nitrendipine binding sites but significantly decreased the affinity (Kd values increased approx. 120%). HPLC investigations have shown that acute treatment with haloperidol and pimozide did not change DA level but produced significant, strong increase the level of dopamine metabolites DOPAC and HVA (by 250% - 400%). Opposite to acute treatment chronic treatment with haloperidol significantly decreased DOPAC and HVA levels in investigated brain structures, whereas chronic treatment with pimozide did not change the activity of DA neurons both 24 h and 9 days after the last dose. The results have shown the differences in mechanism of action between two classic neuroleptics: haloperidol and pimozide.

EFFECT OF ANTIDEPRESSANT DRUGS ON THE LEVEL OF cAMP IN THE NUCLEUS CAUDATUS AND NUCLEUS ACCUMBENS SEPTI OF THE RAT.

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Recently Wachtel presented an overall concept for the etiology of endogenous affective disorders having assumedthe presence of intraneuronal defects at the level of the second messengers function, i.e. beyond the first messengers receptors. Since changes in the dopaminergic (DA) transmission in the mesolimbic system are postulated to play some role in the mechanism of action of antidepressant drugs (ADs), the present study was designed to find out whether prolonged administration of ADs influenced the reactivity of the DA-stimulated cAMP level in the nucleus accumbens septi (NAS) compared to the nucleus caudatus (NC). Wistar rats were treated with imipramine (IMI), oxaprotyline (OXA) or citalopram (CIT). The level of cAMP in slices of the NC and NAS (ex vivo) was measured by RIA. In the NC, lower sensitivity to forskolin stimulation was observed following prolonged administration of ADs, in particular IMI. The use of quinpirole and GppNHp allows a conclusion that a certain up-regulation exists at the level of the inhibitory protein G-i, as well as the D-2 dopaminergic receptor. Similar, or even stronger effects were observed in the NAS. However, other results obtained with D-1 receptor ligands are more complex and do not reveal any common effects of ADs as far as the cAMP generating system is concerned.

IN VIVO VOLTAMMETRIC DETERMINATION OF ENHANCED SPIPERONE-INDUCED RELEASE OF DOPAMINE (DA) AND DIHYDROXYPHENYLACETIC ACID (DOPAC) IN NEOSTRIATUM OF RATS WITH SUPERSENSITIZED DA D_2-RECEPTORS. R. Brus, R.M. Kostrzewa[1], J. Jędrusiak, R. Szkilnik and L. Wójtowicz. Department of Pharmacology, Silesian Academy of Medicine, 41-808 Zabrze, Poland; [1]Department of Pharmacology, College of Medicine, East Tennessee State Univ., Johnson City, TN 37614, U.S.A.

Long-lived DA D_2 receptor supersensitization is produced in rats by ontogenetic quinpirole treatment. To determine whether basal and agonist/antagonist-induced DA and DOPAC release is altered in these rats in adulthood, the following study was performed. Rats were treated daily IP with quinpirole HCl (0.05 mg/kg) or vehicle for the first 11 days from birth. Several weeks later *in vivo* voltammetric recordings were make every 5min for 120min from a working electrode stereotaxically placed in the right striatum. Acute IP injections of SKF 38393 HCl (0.3 mg/kg), quinpirole HCl (0.1 mg/kg), *m*-chlorophenylpiperazine HCl (*m*-CPP: 1.0 mg/kg) and pilocarpine HCl (1.0 mg/kg) - respective DA D_1, DA D_2, serotonin 5-HT_2 and muscarinic-agonists - did not overtly alter DA and DOPAC release in sensitized vs. control rats. Similarly, SCH 23390 HCl (0.3 mg/kg), mianserin HCl (1.0 mg/kg) and scopolamine HCl (0.1 mg/kg) - respective DA D_1, 5-HT_2 and muscarinic antagonists - did not overtly alter DA and DOPAC release in sensitized vs. control rats. The dose of each agonist is known to induced oral activity responses, while the dose of each antagonist is known to inhibit agonist effects. The DA D_2-antagonist, spiperone HCl (0.08 mg/kg), increased DA and DOPAC release for at least 1hr in D_2-sensitized and control rats, but the effect was consistently greater in the D_2-sensitized rats. The enhanced D_2 receptor antagonist-induced release of DA in D_2-sensitized rats indicates that D_2-receptor modulation of presynaptic DA release is altered in these rats. (Supp. by Silesian Academy of Medicine, and Fogarty Intern. Center Health Sci. Exch. with Poland-RMK).

Antidopaminergic activity of EMD 57445, a new selective ligand of sigma receptors

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Some of the sigma receptor ligands are representatives of a promising class of potential antipsychotic agents devoid of extrapyramidal side effects, common to neuroleptic drugs.

In this paper central, especially neuroleptic-like effects of EMD 57445, a sigma ligand with no or very weak affinity for other receptors (including dopamine ones) was studied in rats and mice. EMD 57445 (1-10 mg/kg ip) decreased the locomotor activity in rats and mice. The amphetamine-induced locomotor hyperactivity and stereotypy were decreased by EMD 57445. The behavioural effects of apomorphine, i.e. the locomotor hyperactivity, stereotypy and aggression in rats, as well as climbing in mice, were inhibited by the drug studied. The hyperlocomotion induced by quinpirole (dopamine D2 agonist) and the grooming behaviour evoked by SKF 38393 (D1 agonist) were decreased by EMD 57445. The locomotor activity induced by cocaine, morphine or caffeine in rats and mice were also reduced. EMD 57445 inhibited the behavioural stimulation induced by non-competitive (MK-801) or competitive (CGP 37849) NMDA receptor antagonists. EMD 57445 neither induced catalepsy nor increased the muscle tone in rats.

The results suggested that EMD 57445 may be a useful as an antipsychotic drug devoid of extrapyramidal side effects.

Poster sessions - Neuroanatomy

NEURONS OF THE CLAUSTRUM PROJECTING TO THE SOMATOSENSORY AND MOTOR CORTICAL AREAS IN THE RABBIT

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Connections of the claustrum with the neocortex have been studied extensively in some species. However, the claustocortical connections of the rabbit are still unknown in spite of the fact that this species is often used for experimental studies on the physiology of subcortical nuclei. The aim of our study was to define the localization and characteristic features of claustral neurons related to the motor and somatosensory systems of the rabbit.

The experimental material consisted of 15 New Zealand rabbits. The connections of claustrum with the somatosensory and motor cortex were studied by means of fluorescent tracers (FluoroGold, Nuclear Yellow, Fast Blue), administered intraoperatively to stereotactically verified target areas in various regions of the motor and somatosensory cortex. Overlapping of the somatosensory and motor regions was verified by means of two different tracers, administered simultanously to these regions.

Neurons which project to the motor cortex were localized mainly in the anterior part of the claustrum. Additionally, dispersed labelled cells were observed in the central part; no labelled cells were found in the posterior part of the claustrum. Neurons which project to the somatosensory cortex were identified in the central part of the claustrum, in its dorsal portion. Single fluorescent cells were also observed in more anterior and posterior parts. However, the anterior and posterior poles of the claustrum were free of them. The area of the most densely packed labelled cells was surrounded by the area of rather scattered ones.

LOCALIZATION OF THE VISUAL AREA IN THE CLAUSTRUM OF THE RAT, RABBIT AND CAT

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It has been shown that the claustrum has connections with the visual cortex. In the cat and monkey the visual area of the claustrum is even retinotopically organised. However, in other animals the relationship of the claustrum to the visual system is still unknown.

The projections of the claustrum to the visual cortex were studied in eight New Zealand rabbits, three Wistar rats and three cats. Fluorescent retrograde tracers were used (FluoroGold, Nuclear Yellow and Fast Blue). Under general anaesthesia the tracers were injected into the visual cortex. The injection sites were verified on the slices according to the cytoarchitectonic characteristics of the cortical visual region. The animals were perfused 48 hours later and the frozen brains were cut into 40-μm-thick slices which were studied under a fluorescent microscope. We established the percentage of neurones labelled with the tracers along the rostrocaudal axis as well as their mediolateral and dorsoventral distribution.

We found that in all the species examined claustrocortical visual connections originate mainly in the posterior part of the claustrum. In the rat the labelled cells were also found in the central part. In the anterior part only single labelled cells were observed. In the rabbit's claustrum the labelled cells were also localized mainly in the posterior part. In the central part only single cells containing fluorescent tracers were present; however, in this part they were much less numerous in the rabbit than in the rat. In the cat the labelled neurones were localised almost exclusively in the posterior part of the claustrum.

Comparing the claustral visual area of the rat, rabbit and cat we may conclude that its localisation is the most concentrated in the cat, whereas in the rat there is an evident overlap with the somatosensory area situated more rostrally.

ORGANIZATION OF CLAUSTRONEOCORTICAL CONNECTIONS IN THE RAT

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The organization of claustroneocortical connections was studied in 20 Wistar rats (both sexes) by means of retrogradely transported fluorescent tracers (Diamidino Yellow, FluoroGold, Nuclear Yellow, Fast Blue) injected into the motor, somatosensory or visual cortical fields. After 48 hour survival, the animals were sacrificed with a lethal dose of sodium pentobarbital and perfused with heparinized saline, followed by cacodylate-buffered 4% formalin. The brains were cut frontally into 40-µm-thick frozen sections. Every third section was stained with cresyl violet. The density of retrogradely labeled neurons was measured in each section and compared with the density of neurons in the neighbouring section stained with cresyl violet.

After injection into the motor cortex up to 80% of all the neurons in the section was retrogradely labeled in the rostral part of the ipsilateral, insular claustrum. In the central part of the claustrum the number of neurons containing fluorescent tracer was much lower (about 20%). The injections into the somatosensensory cortex resulted in abundant labeling in the central part of the ipsilateral insular claustrum (up to 60%). The number of labeled cells in the rostral part was lower (up to 20%) whereas in the caudal part it was the lowest (less than 10%). After the injection into the visual cortex the number of labeled neurons reached 50% in the caudal part of the claustrum, whereas in the central part - only 15%. In the most rostral portion of the claustrum the cells containing fluorescent tracer were not present. In the contralateral insular claustrum the topographical distribution of labeled neurons was similar to that observed ipsilaterally but their number was much lower (up to 15%). In the prepiriform claustrum retrogradely labeled cells were not found.

The following conclusions may be drawn from our studies: (1) The rat insular claustrum possesses strong projections to the motor, somatosensory and visual cortical fields. (2) The organization of claustroneocortical connections shows the specific anteroposterior topographic distiribution. Neurons in the rostral part of the claustrum mostly project to the motor cortex, those in the middle part - to the somatosensory cortex, whereas axons of neurons localized in the posterior part terminate in the visual cortex. (3) The above groups of corticopetal neurons show a moderate degree of overlap.

Chemical coding of cholinergic neuronal subdivision in the porcine paracervical ganglion and its projections

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Numerous physiological and pharmacological studies strongly support the hypothesis, the female paracervical ganglia (PCG) comprise a parasympathetic (cholinergic) neuronal subpopulation, however, due to the lack of antibodies recognizing the choline acetyltransferase (ChAT) in the peripheral nervous system, there is no direct evidence for the real nature of the "putative cholinergic" (i.e. acetylcholinesterase-positive) pelvic neurons. As recently an antibody against ChAT, working well in the periphery, has been raised, we aimed the present study at disclosing the occurrence and intraganglionic distribution of ChAT-immunoreactive (ChAT-IR) structures within the porcine PCG. Moreover, the co-localization of ChAT with tyrosine hydroxylase (TH), vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), substance P (SP), somatostatin (SOM) and Leu^5-enkephalin (LENK) was investigated by means of a double-immunofluorescence technique. ChAT-immunoreactivity was found in the vast majority of PCG neurons, however, there were differences in the type of chemical coding of particular perikarya: a large proportion of ChAT-IR neurons co-exhibited immunoreactivity to VIP and/or NPY, while only in a small numbers of ChAT-positive perikarya were simultaneously SOM- or LENK-IR. In addition to the perikarya, numerous linear and varicose ChAT-IR nerve fibres forming moderate to dense meshes around ChAT-IR or non-reactive neurons (often in a basket-like manner) were also observed. ChAT-IR neurons received a sparse LENK-, SOM- or VIP-IR nerve supply, however, they were well supplied by SP-IR varicose fibres. Retrograde tracing revealed, that both the porcine ovary and uterus receive their cholinergic nerve supply from the paracervical ganglia. Retrogradely labelled, cholinergic neurons exhibited various combinations of immunoreactivities to VIP, NPY, SOM and LENK; they were also supplied by numerous ChAT-, SP- or LENK-IR varicose terminals. The present findings confirm that cholinergic neurons indeed exists in paracervical ganglia and provided direct evidence for the co-occurrence of VIP, NPY, SOM or LENK with ChAT in pelvic parasympathetic neurons supplying the ovary and uterus.

CONNECTIONS OF THE SUPERIOR TEMPORAL CORTEX WITH THE POSTERIOR THALAMUS AND THE AMYGDALA IN RHESUS MONKEY.

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Auditory association areas of the superior temporal cortex (AA1, AA2, AA3) were injected with fluorescent tracers (FB, DY, FR) in three monkeys. Data showed that each of the areas receives projections from the non-tonotopic, dorsal and medial parts of the medial geniculate body (MGB). However, AA2 receives significant projections from the dorsal (d) and suprageniculate-limitans (Sg-Lim) nuclei, whereas AA3 mainly from Sg-Lim. FR injections into AA2 and AA3 revealed also a geniculopetal projection terminating in the posterodorsal and medial parts of MGB as well as in Sg-Lim.

Each of the associations areas is reciprocally connected with the thalamic medial pulvinar nucleus (Pm), but they receive projections from different populations of the pulvinar neurons.

The lateral, basal lateral and basal accesory amygdaloid nuclei send strong, corticopetal projection to AA3 and weaker to AA2. In turn AA2 and AA3 project to the lateral amygdaloid nycleus.

The findings suggest that in auditory processing to amygdala, AA2 and AA3 areas cooperate with both association MGB nuclei and the lateral amygdaloid nucleus.

ULTRASTRUCTURAL AND MORPHOMETRIC FEATURES OF NEURONS OF THE CAT'S VISUAL CLAUSTRUM

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The claustrum is a telencephalic structure abundantly connected with the cerebral cortex. It has been generally accepted that in the claustrum there are intrinsic neurons and neurons projecting to the neocortex and forming the ascending limb of the claustrocortical neuronal loop. The aim of the study was to characterize morphometric features of claustral cells and to differentiate between projective and intrinsic neurons with the retrograde transport method.

Eight adult cats were used. In three of them, the horseradish peroxidase (HRP or WGA-HRP) was injected streotaxically into the primary visual field. The other five cats were used for morphometric study. All the animals were perfused through the heart with paraformaldehyde and glutaraldehyde. Next, specimens taken from the visual claustrum were post-fixed in osmium tetroxide. Ultrathin sections were examined with a JEM 1200 EX II electron microscope. Retrograde labelled neurons were analyzed both with light and electron microscope on the basis of electron-dense material localized in the perikarya.

Three groups of neurons were distinguished: small (<150 μm^2); medium-sized (151-250 μm^2) and large ones (>250 μm^2). In each group the shape of perikaryon and nucleus, nucleus/cell body area ratio as well as the number of synaptic contacts were taken into account. Retrogradely labelled with HRP or WGA-HRP neurons of the claustrum were mostly medium-sized with wide proximal dendrites; large labelled cells were also found. We observed neither HRP or WGA-HRP labelling of small neurons.

The present results support the hypothesis that mainly medium-sized and larger neurons are projective and form the ascending limb of the claustrocortical loop, whereas small ones with narrow proximal dendrites are probably predominantly intrinsic.

Projection of the basal forebrain region upon the temporal cortex in the dog.

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The present study analyzed the basal forebrain (BF) projection to the temporal cortex (TCx) in the dog by using combined tract-tracing and immunohistochemical techniques. On the basis of cytoarchitectonic criteria, the dog BF complex consisted of the medial septal nucleus nuclei of the diagonal band of Broca, magnocellular preoptic nucleus, substantia innominata, and the basal nucleus proper (MBN). The spatial organization of ChAT-immunoreactive neurons of BF basically agrees with that described cytoarchitectonically. To analyze the fibre projection from BF to the temporal cortex, dogs received an intracortical injections of fluorescent retrograde tracers. Retrogradely labeled neurons that, in some percentage, colocalized also ChAT and AChE were found predominantly in the posterolateral part of BF corresponding to the basal nucleus. Neurons that exhibit retrograde labeling were mainly discerned after the injections in the auditory associative areas of the TCx. Following the injections in the auditory sensory areas much fewer labeled cells in MBN were observed. It seems that the projection from MBN to the auditory cortex of the temporal lobe become progressively restricted to the associative areas. These data are discussed in terms of the putative role the cholinergic input might play in cognitive processing.

VISUOTOPIC ORGANIZATION OF PROJECTIONS FROM THE PULVINAR COMPLEX TO PRESTRIATE CORTEX (V2) IN MACAQUE MONKEYS.
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Previous studies in macaques demonstrated that both inferior (PI) and lateral (PL) divisions of the pulvinar are reciprocally connected with the entire occipital lobe, including prestriate cortex (V2). As the results of recent architectonic studies suggest, the inferior pulvinar may consist of four distinct parts or nuclei. The purpose of present study was to determine the pattern of connections between V2 and particular subdivisions of the pulvinar in macaque monkeys (*M. mulatta , M. fascicularis*). Multiple injections of up to 5 tracers (fluorescent dyes and WGA-HRP) were made in dorsal and ventral V2, representing respectively lower and upper central vision. After about one week survival, deeply anesthetised animals were perfused, and thalamic blocks were cut into 40-50 um frozen sections. Transported label was related to architectonic subdivisions of pulvinar delineated on the basis of adjacent Nissl, CO and AChE-stained sections. Injections in dorsal and ventral V2 labeled neurons in both, lateral and inferior divisions of the pulvinar. Separate groups of label in PI suggest existance of at least two PI subdivisions: central (Plc) and medial (Plm). These subdivisions can be also distinguished architectonically, especially in the AChE pattern. Arrangement of thalamocortical connections showed that PL, Plc, and perhaps Plm are retinotopically organized. Relating the location of label in the thalamus to the retinotopic locations of injection sites in V2 indicates that the foveal representation is located laterally in PL and Plc, while succesively more peripheral parts of contralateral hemifield are represented more medially, with the lower visual quadrant represented dorsally and the upper visual quadrant represented ventrally. In each animal, the representation of the lower visual field is more rostral, while that of the upper field is more caudal. (Supported by NEI Grant EY-O2686)

Poster sessions - Varia

ABSTRACT FORM

BOT IT2, NEW INSECT TOXIN MODIFYING THE ACTIVITY OF INSECT AXONAL SODIUM CHANNELS.

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Several "excitatory" insect toxins isolated from different venoms of Old World scorpions induce in insect axonal membranes a limited depolarization accompanied by repetitive spike activity. In voltage clamp they maintain or increase the Na^+ peak current and slow its decreasing phase in a voltage dependent manner or induce the development of a voltage sensitive delayed Na^+ conductance. Similar effects, in current clamp, were obtained in Ranvier node by β toxins isolated from the New World scorpion venoms. β toxins affecting mainly Na^+ activation result in the decrease of Na^+ peak current and in the development of new Na^+ inward current after the end of each depolarizing pulse.

In our study, action of new insect toxin Bot IT2, (gift of L. Borchani, Pasteur Inst.,Tunis) isolated from the Tunisian scorpion, Buthus occitanus tunetanus venom on the cockroach giant axon has been observed. Bot IT2, at a concentration 10^{-7} mol/l induces limited depolarization and develops depolarizing afterpotential, repetitive activity and later, plateau potential terminated by bursts. In voltage clamp, after Bot IT2, a decrease of the Na^+ peak current simultaneous with the development of a new current very slowly activated-deactivated kinetics has been observed. Voltage dependence of this slow current is not significantly different from that of the control current. The results suggest that BotIT2 modifies the kinetics of axonal membrane Na^+ channels activation and probably transforms the normal, fast Na^+ channels into slow ones. Obtained results indicate also that "excitatory" toxins modify both activation and inactivation of Na^+ channels.

More general conclusions are: 1. Different modifications of Na^+ channel function can result in the same pattern of neuronal activity; 2. "excitatory" toxins of the Old World scorpion venom close correspond to β toxins (described as active on vertebrates) in the venom of New World scorpions.

EFFECTS OF EXTRACELLULAR HIGH OSMOTIC PRESSURE ON K^+ CURRENT IN INSECT AXONAL MEMBRANE.

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K^+ accumulation as a result of neuronal activity is observed as well in central as in pheripheral nervous system. It has been demonstrated that presence of K^+ accumulation causes difficulties to precise the characteristics of K^+ conductance in insect giant axon. Changes of $[K^+]$ in periaxonal space induce a shift of reversal potential for the K^+ current and can have a direct effect on K^+ channel. Application of patch clamp technique for observation of K^+ channel function in insect giant axon failed till now due to Schwann cell layers surrounding axonal membrane. In squid axon, K^+ accumulation was removed by an outward water flow created by an osmotic gradient across the axolemma. In the present work, K^+ current was observed in cockroach isolated giant axon using double oil gap technique in conditions of high osmotic pressure in extracellular fluid induced by application of urea and glucose.

Effects of urea depended on its concentration. 0.5 mol/l induced a decrease of K^+ accumulation concomitantly with the progressive increase of outward K^+ current. In 5 min a new equilibrium state was obtained and further changes were not observed. The effect of 1 mol/l urea was the same at the begining (step I) but later the increase of K^+ current was associated with the increase of K^+ accumulation (step II). Time course of K^+ outward current has not been changed in the presence of urea. Effect of glucose (0.3, 0.5 mol/l) appeared at similar steps as after 1 mol/l urea. However, compared to effect of urea the increase of K^+ outward current in step I was much smaller and removal of K^+ accumulation more marked, new equilibrium state has never been observed. In step II the increase of K^+ current was continued to the death of axon and important change of time course of K^+ current has been observed. Reversibility in physiological saline was much smaller than after urea.

Presented results are preliminary but it is clear that: 1.high extracellular osmotic pressure decreases K^+ accumulation; 2.the increase of outward K^+ current in not attributed only to the decrease of K^+ accumulation; 3. osmolal effect depends on the size of used molecule.

THE EFFECT OF REPEATED AMPHETAMINE ADMINISTRATION ON STEROID HORMONE LEVELS IN THE RAT PLASMA

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Steroid hormones and some of their derivatives exert a profound influence on the central nervous system activity via both genomic and non-genomic mechanisms. Furthermore, steroid hormones modulate effects of psychomotor stimulants: for example, progesterone and testosterone enhance and inhibit the amphetamine-induced effects, respectively. In the present study we investigated effects of repeated amphetamine administration (5 mg/kg, i.p., twice a day for 14 days) on the concentration of gonadal and adrenal steroids in the plasma of male Wistar rats. The obtained results showed that repeated amphetamine had no significant effect on the progesterone and testosterone levels, but attenuated the concentration of the GABA-receptor modulator androstenedione at 24 hours after the last injection. On the other hand, the corticosterone level was increased in the plasma of rats chronically treated with amphetamine. The above results indicate that repeated amphetamine influences the plasma levels of some neuroactive steroids, which may contribute to the amphetamine-induced changes in neuronal excitability.

INFLUENCE OF CHRONIC HYPERGLYCAEMIA ON THE MODULATION OF SYNAPTIC ACTIVITY IN THE HIPPOCAMPUS BY ANOXIA/AGLYCAEMIA

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During brain hypoxia there is a significant increase in the extracellular levels of adenosine which has been shown to mediate the initial fast but reversible failure of synaptic transmission in the CA1 region of the hippocampus. Application of adenosine attenuates ischaemic neuronal damage and it is believed that synaptic inhibition contributes to adenosine's neuroprotective actions by preventing energy consuming signalling.

Diabetes is an important risk factor in stroke, not only are diabetics more likely to suffer from a stroke but hyperglycaemia greatly increases stroke related neuropathology and reduces chances of recovery. Paradoxically, however, it has recently been shown that chronic hyperglycaemia in the rat increases the sensitivity of hippocampal pyramidal neurones to adenosine. We have, therefore, investigated how an increase in adenosine sensitivity shapes the electrophysiological response of the hippocampus to anoxia/aglycaemia (A/A) in chronically hyperglycaemic rats.

Experiments were performed on transverse hippocampal slices from rats made diabetic (blood glucose≥20mM) by injection of streptozotocin. Evoked field potentials were recorded from the stratum radiatum of the CA1 region following stimulation of the Schaffer collateral-commissural pathway. A/A was induced in slices by changing the oxygenating gas to 95%N_2,5%CO_2 and replacing the glucose in the ACSF with sucrose.

Adenosine application inhibited evoked synaptic potentials in a dose-dependent manner but as expected slices from hyperglycaemic rats showed an increased sensitivity. AA-induced inhibition of synaptic transmission was also enhanced and occurred earlier in hyperglycaemic rats. This change in the rate of synaptic inhibition was abolished by application of A1 receptor antagonists, consistent with it being mediated by AA-induced adenosine build up in the tissue. The significance of these findings and the role of hyperglycaemia-induced increase in adenosine sensitivity with respect to overall neuronal viability after oxygen deprivation remains to be investigated.

This work was supported by the Wellcome Trust

EXCITATORY EFFECTS OF THE 5-HT4 RECEPTOR ACTIVATION IN THE HIPPOCAMPUS ARE ALTERED BY CHRONIC TREATMENT WITH IMIPRAMINE.

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Serotonin (5-HT) induces a hyperpolarizing response which is followed by depolarization and a decrease in slow afterhyperpolarization (AHP)in pyramidal cells of the CA1 region of the hippocampus . The hyperpolarizing response has been shown to be mediated by 5-HT_{1A} receptors, while the receptor that mediates slow excitatory responses to 5-HT has a pharmacological profile similar to that of 5-HT_4 receptors. We tested the effects of the partial 5-HT_4 receptor agonist zacopride on CA1 neurons in hippocampal slices using intracellular and extracellular recordings. Zacopride (5-10 μM) blocked the cell discharge adaptation and AHP. The amplitude of population spikes elicited by stimulation of the Schaffer collateral/commissural fibers was increased in the presence of zacopride, that effect being significant in the case of low stimulation intensities only. In the hippocampal slices prepared from rats which had been treated for two weeks with the serotonin and norepinephrine uptake inhibitor imipramine, the effect of zacopride on the population spikes was significantly diminished. Our previous studies demonstrated that prolonged treatment with imipramine enhanced the effect of the 5-HT_{1A} receptor activation in the hippocampus. Thus the antidepressant drug imipramine induces supersensitivity to the inhibitory effect of 5-HT and subsensitivity to the excitatory effect of 5-HT, the latter effect being mediated by 5-HT_4 receptors.

The Involvement of Central Adrenergic Mechanism in the Pituitary-Adrenocortical Response to Cholinergic Stimulation.

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Cholinergic neurons modulate the hypothalamic-pituitary-adrenal (HPA) axis, predominantly via stimulation of hypothalamic release of corticotropin-releasing hormone. Although a cholinergic mechanism itself may be involved in the modulation of ACTH secretion, it seems possible that cholinergic agonist-induced changes in the hypothalamic monoaminergic neuronal activities are implicated in modulating ACTH and corticosterone secretion.

In the present experiments the significance of central adrenergic system in activation of the HPA axis by cholinergic stimulation was investigated in rats. Cholinergic agonist carbachol (2 μg), administered into the lateral cerebral ventricle of conscious rats induced a significant rise in the serum corticosterone levels 1 h later. This increase was totally abolished by icv pretreatment 15 min earlier with atropine.
Prazosin (0.1 μg), an α_1-adrenergic receptor antagonist, and propranolol (10 μg), a β-adrenergic antagonist, significantly impaired the corticosterone response to carbachol, whereas yohimbine (1 μg), an α_2-adrenergic antagonist, only moderately diminished the increase in corticosterone secretion elicited by carbachol. Lesions of central noradrenergic terminals by a selective noradrenergic neurotoxin DSP-4, 50 mg/kg ip 8 days prior to carbachol, considerably reduced both the hypothalamic noradrenaline level and carbachol-elicited corticosterone response.

These results indicate that central noradrenergic neurons and adrenergic receptors are significantly involved in activation of the HPA axis by the cholinergic system.

Clinical Evaluation of H_1-Receptor and H_2-Receptor Antagonists for Acute Postoperative Pain. C.W. Berthold and R.A. Dionne, Clinical Pharmacology Unit, NAB, NIDR, NIH, Bethesda, MD and Department of Pharmacology, LSU Medical Center, New Orleans, LA 70112.

Postoperative pain following surgery is believed to be primarily due to acute inflammation. Mediators thought to modulate acute pain include bradykinin, prostaglandins, serotonin, substance P, and histamine. Both clinical and animal studies have provided evidence that histamine is a mediator for cutaneous pain. The present study evaluated the analgesic effects of peripherally-acting antihistamines (H_1 and H_2 receptor blockers) in the oral surgery model of pain. A double-blind, placebo-controlled study compared the analgesic activity of an H_1 antihistamine, terfenadine 60 mg, and an H_2 antihistamine, ranitidine 150 mg, to ibuprofen 600 mg and placebo. Drugs were given to a total of 127 patients an hour before oral surgery. Analgesia was assessed every 30 min for 240 min following surgery. Analgesic efficacy was compared using standard pain scales: visual analog scale (VAS), category scale, graphic rating scale and global rating scale of pain relief. Pain increased over time from 90 to 240 min in all groups as measured on the VAS. An ANOVA of VAS scores over 90 to 240 min showed a significant difference between groups ($F(3,122)=19.65$, $P<0.0001$). Post hoc comparisons showed that the mean pain intensity for the VAS from 90 to 240 mins was significantly less for ibuprofen ($p<0.01$ all others). Terfenadine and ranitidine failed to produce any analgesia and were comparable to placebo. Similar differences between groups were observed for ANOVA of the graphic rating scale ($F(3,122)=19.24$, $p<0.0001$) and nonparametric analysis of the category scale (Kruskal Wallis statistic = 26.26), $p<0.0001$, and the global rating of pain relief ($KW=26.26$, $p<0.001$). These data indicate that pretreatment with a single dose of an antihistamine specific for either the H_1 or H_2 receptor does not produce analgesia in the oral surgery model suggesting that antihistamines acting primarily at peripheral sites are devoid of analgesic activity.

ACTIVITY OF INSECT TOXINS FROM THE BUTHUS OCCITANUS TUNETANUS VENOM.

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A scorpion crude venom comprises a number of polypeptide toxins strikingly affecting Na^+ permeabilities of excitable membranes. Some of them demonstrate high specificity to insect neuronal membrane. They are valuable tools for studying the function of insect Na^+ channels in order to discover insect specific targets for new highly selective molecules useful for insect control. From the venom of the Tunisian scorpion Buthus occitanus tunetanus (Bot) some fractions specifically active against insects have been discovered lastly (El Ayeb M.,Inst.Pasteur,Tunis). Their effects on insect axonal membrane have been studied. Experiments were performed on isolated cockroach giant axon using double oil - gap method in current and voltage clamp conditions.

Fraction Bot IT1 induces prolongation of evoked action potentials to plateau potentials. Similarly to α toxins, Bot IT1 slows inactivation of Na^+ current but does not affect its deactivation. Bot IT4 and Bot IT5 slowly and progressively depolarize the axonal membrane and block evoked action potentials. Resting depolarization can be supressed by TTX. In voltage clamp, progresive block of Na^+ current is concomitant with the development of a discrete inward resting current, sensitive to TTX.

The conclusion is that the venom of Bot contains toxins differently affecting insect Na^+ channels but it remains to be seen which differences in their structure are responsible for their effects and their selectivity to insect neuronal membranes.

RESPONSES OF SINGLE RENAL SYMPATHETIC POSTGANGLIONIC FIBRES TO LIMINAL STIMULATION OF RENAL AFFERENTS IN THE RABBIT

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The studies of renorenal reflexes were mainly concerned with responses to repetitive stimulation. We have investigated the effects of liminal stimulation of afferent C fibres in the renal nerve on spontaneous sympathetic discharge in single ipsilateral renal postganglionic fibres. In rabbits anaesthetized with urethane + chloralose two bundles of fibres in the left renal nerve were identified. One bundle was employed for single fibre recordings of sympathetic postganglionic discharge.. The other bundle was used to stimulate afferent C fibres with single pulses or short trains of pulses (2-3) at 10 Hz. Clear-cut changes in peri-stimulus time histograms of sympathetic discharge were observed in 10 out of 11 recorded fibres. Inhibitory responses consisting in transient suppression of sympathetic discharge were produced by single pulse in 1 fibre, by two pulses in 4 fibres and by three pulses in 2 fibres. The increase in the number of shocks did not affect the latency of the inhibitory responses (which ranged from 178 to 233 ms) but substantially lengthened the duration of inhibition of sympathetic discharge (from 193 to 653 ms). Excitatory responses consisting in acceleration of sympatheic discharge were observed in 3 fibres. They were evoked by 3 pulses. The latency of these responses was 447 ± 23.8 ms ($x \pm$ S.D.) and their durations amounted to 143 ± 93.8 ms. These findings show that responses of single renal postganglionic fibres to activation of renal C afferents exhibit low threshold which seems to be only slightly higher than that of responses to stimulation of aortic C fibres.

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IMPAIRMENT OF THE NERVOUS SYSTEM IN SEPSIS;

Patients suffering from nervous system impairment by sepsis have been studied: 91 babies, pre-school and school age children and 103 adults. Sepsis causes lesions in the central, as well as in the peripheral nervous system. However, lesions of this etiology in children and in adults possess certain distinguishing peculiariries. In children, impairement of the nervous system is characterized by the development of non-specific neurologic syndromes: meningism, liquorodynamic disorders, brain oedema, cramps, vegetovascular disorders, the hyperthermal syndrome, cerebral coma. Following meningoencephalites and encephalopathies, younger children in sepsis not infrequently develop apallic disorders manifested as the decortation and the cerebration syndromes of the "vegetative condion". These syndromes are more often observable in premordibly aggraveted children. In exceptional cases, still younger septic children suffering from meningoencephalites and encephalopathies also develop disorder of the thermoregulatory centre, and this, in sleep, is manifested by the development of poikilothermic conditions. In a number of cases, this syndrome is combined with respiratory disorders, which in such situations is an alarming complication. In septic babies with pronounced manifestations of intoxication, signs of meningism sometimes emerge against the backgroung of low intracranial pressure. Septic adults develop meningites, meningoencephalites, sinus thromboses, pseudoinsults, subarachnoid haemorrhages, encephalopathies, and lesion of the peripheral nervous system. In obstetric-gynaecologic sepsis, the development of cerebral sinus thromboses and cerebral thrombophlebites proceeds by several ways, among others it follows retrogressively Batson's venous network.

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Correlation of Magnetic Resonance Imaging, Hormonal Homeostasis and Neurophathological Conditions due to Ischemic Brain Injury.

In the present study we examined the functional state of hormonal homeostasis of 57 patients with mood disturbance after brain injury. The neurological examination and diagnosis were done according to the criteria established by the Stroke Data Bank (US,NIH, 1984). All patients also were examined by psychiatrist for several times. Magnetic resonance imaging (MRI) makes possible to compare the level of brain injury with results of clinical, radioimmunoassay and biochemical investigations (the blood content of cortisol, prolactin, adrenaline, noradrenaline and adrenocorticotrope hormone).

On the basis of our laboratory findings we have postulated that association between poststroke mood disorders and the hemisphere in which the lesion is located (i.e. between depression and left hemisphere injury and between undue cheerfulness and right hemisphere injury) might be based on different emotional-behavioral response of hemispheres In other words the ishemic injury of different hemispheres produse different biochemical response due to damage of brain (the degree of disturbances of hormonal homeostasis was higher in right hemisphere injury).

Supravital studies of effects of proteases on injured and uni-injured nerve fibres

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Not received

Motor recovery after hemiparetic stroke: relation to pyramidal tract damage and thalamic hypometabolism.

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We examined whether the patterns of depressions of the regional cerebral glucose metabolism (rCMRGlu) are of prognostic value for motor recovery in first hemiparetic stroke. Specifically, we related the course of the clinical and electrophysiological abnormalities during four weeks after stroke to the changes of the structural stroke lesions and to the associated rCMRGlu depressions. Subjects and methods: 25 patients of both sexes (mean age 56 ± 13 (SD) years) with first hemiparetic stroke were entered into the study. Motor impairment in the acute stage and motor recovery in the early chronic stage were assessed clinically using a motor score and electropysiologically by magnetic evoked motor potentials (MEP) recorded from the contralateral first dorsal interosseus muscles. In addition, the spatial extents of the underlying structural brain lesions were determined planimetrically on proton-weighted magnetic resonance (MR) images at the two stages. Correspondingly, the proportion of the pyramidal tract damage was quantified after superimposition of MR images on the Talairach and Tournoux atlas (1988). The resting rCMRGlu was measured with PET on average 21 days after infarction as detailed elsewhere (Seitz et al. 1994). Results: Patients with good and poor outcome showed no significant differences in the sizes of their acute brain lesions. In contrast, patients with a significant restitution of motor functions had smaller brain lesions ($p < 0.02$) in the early chronic stage than in those with poor improvement of their motor impairments. Location of the brain lesion was a major determinant for motor recovery: correlation analysis revealed that recovery of maximal grip force and dexterity were inversely related to the degree of pyramidal tract damage as determined by planimetry of MR images and by MEP recordings. PET data revealed significant rCMRGlu depressions in the thalamus ipsilateral to the brain lesions ($p < 0.01$). The figure shows that the rCMRGlu depression in thalamus relative to the contralateral side correlated with the relative reduction of MEP amplitudes ($r = 0.90$) and the degree of the structural pyramidal tract damage ($r = 0.85$) separating patients with good (white columns) and poor (black columns) outcome. In contrast, the spatial extent of the rCMRGlu depressions induced by the stroke lesion did not correlate with the acute motor disturbances or the thalamic hypometabolism.
Conclusion: Our morphometric data provide evidence that not the size of the stroke lesion but the location relative to the pyramidal tract was the major determinant for motor impairment in hemiparetic stroke. The differences of remote metabolic depressions in the ipsilateral thalamus among patients with good and poor outcome suggest that in addition to pyramidal tract damage the integrity of the thalamic plays a significant role for motor recovery.

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