MOLECULAR BASIS OF NEURAL FUNCTION

Edited by
S. Tuček
S. Štípek
F. Šťastný
and
J. Křivánek

EUROPEAN SOCIETY FOR NEUROCHEMISTRY

MOLECULAR BASIS OF NEURAL FUNCTION

Abstracts of the Sixth ESN General Meeting in Prague 1986

Edited by S. Tuček, S. Štípek, F. Šťastný and J. Křivánek

MOLECULAR BASIS OF NEURAL ACTIVITY

Abstracts of the Sixth General Meeting
of the European Society for Neurochemistry

Edited by S. Tuček, S. Štípek, F. Šťastný and J. Křivánek

EUROPEAN SOCIETY FOR NEUROCHEMISTRY
PRAGUE 1986

International Standard Series Number ISSN 0862-0385

AUTORADIOGRAPHIC MAPPING OF NEUROTRANSMITTER RECEPTORS IN THE NORMAL AND PATHOLOGICAL HUMAN BRAIN

J.M. Palacios[1]*, R. Cortés[1], A. Probst[2], M. Dietl[1]
(1) Preclinical Research, Sandoz Ltd. and (2) Institute of Pathology, Dept. of Neuropathology, University of Basle, Basle, Switzerland.

Neurotransmitter receptors in the human brain have been extensively characterized using biochemical methods (this symposium). One of the main limitations of these methods is its limited anatomical resolution. The use of in vitro autoradiographic methods allows the quantitative study of receptors with the resolution of the light microscope. This methodology has been applied extensively in our laboratory and by others to the study of receptors in normal and pathological human postmortem brain tissues (1,2).

The influence of factors such as premortem condition, postmortem delay, age, sex, drug treatment, etc. which could affect receptor properties was examined first. While postmortem delays appear to have little influence on the density and distribution of receptors in postmortem brain tissue, sex and age influence receptor densities in a variety of manners and also differently in the various brain regions.

The pharmacological and anatomical similarities and differences between human brain and rat brain, the most commonly used laboratory animal, were examined afterwards. Close pharmacological and anatomical similarities were seen for some receptors, for example the cholinergic muscarinic. Others, for example alpha$_1$-adrenoceptors, presented comparable pharmacology with marked anatomical differences. Finally, both anatomical and pharmacological differences have been seen for other receptors. Species differences are an important characteristic of brain receptors and deserve further investigators.

Finally, many efforts have been directed to the investigation of receptor changes in brain pathologies particularly in several degenerative diseases such as amyotrophic lateral sclerosis (ALS), Huntington's chorea (HD), Parkinson's disease and Alzheimer's disease. At least two types of receptor alteration have been found in pathological tissues: 1) Receptor changes directly related to the neuronal losses associated with these diseases. Examples are receptor losses in the ventral horn of the spinal cord of ALS patients or in the caudate in HD. 2) Most interestingly changes which are not directly related to cell loss or that even precede neuronal death have also been observed. For example some receptors are altered in HD even in early stages of the disease before cell loss and atrophy are detectable (3).

Receptor autoradiography techniques are then providing information which combined with the newly developed methods for non-invasive visualization of receptors in the living man will in the near future largely influence our current ideas on receptor mechanisms in the human brain.

1) J.M. Palacios et al. (1986) Trends in Neurosciences 9, in press.

2) P.J. Whitehouse (1985) Trends in Neurosciences 8, 434-437.

3) Walker et al. (1984) Neurology 34, 1237-1240.

CCK PHARMACOLOGY IN RAT DURING INTRACRANIAL SELF STIMULATION. CCK BINDING SITES IN RAT AND HUMAN LIMBIC STRUCTURES STUDIED BY AUTORADIOGRAPHY

J-J. VANDERHAEGHEN[1], S. GOLDMAN[1], C. HEIDBREDER[2], O. VAN REETH[1], D. PELAPRAT[3], B.P. ROQUES[3] AND Ph. DE WITTE[2], [1]Université Libre de Bruxelles and [2]Université Catholique de Louvain, Belgique. [3]Université René Descartes, France.

CCK is co-localised with dopamine (DA) in some VTA neurons projecting through the medial forebrain bundle to the caudal part of the accumbens and is heavily represented in the limbic system. CCK 8 has been reported to have neuroleptic like properties and we have shown low levels of CCK in human schizophrenic CSF. CCK-8 inhibited DA activity after ventricular (ICV) or peritoneal (IP) injection althought it stimulated DA neurons when iontophoretically injected into the ventral tegmental area (VTA). Using intracranial self stimulation paradigm with an electrode placed in the hypothalamus near the medial forebrain bundle, we observed in the rat an inhibition (IP, ICV or rostral accumbens) or a potentiation (caudal accumbens) of self stimulation depending on the route and site of CCK-8 injection. Using ^{125}I-Bolton-Hunter CCK8 as ligand, we observed some similarities in CCK binding sites distribution in rat and human limbic brain structures. The incubation media contained 10^{-10} M ^{125}I-CCK8 and for the estimation of non specific binding, 10^{-6} M unlabeled CCK8. CCK binding sites are largely represented in the limbic structures with some variation in affinity regarding the regions. CCK 8 binding sites are of lower affinity in the caudal than in the rostral part of the accumbens in the rat. Similarly, in the human, the binding is more important in the caudate (maximum), putamen and rostral accumbens than in caudal accumbens. The subiculum, the frontal lobe and the amygdaloid complex but not the hippocampus contains large amounts of binding sites. CCK is of importance and of potential interest in limbic system pharmacology and pathology in the rat and the human.

Supported by the Belgian FRSM 34521.82 and 34523.86, and the "Fondation Médicale Reine Elisabeth" 1986. S.Goldman and O.Van Reeth are fellows of the Belgian FNRS.

[^{3}H]NICOTINE BINDING TO HUMAN BRAIN: A DEVELOPMENTAL STUDY.

Sarah Latham, Susan Wonnacott
Department of Biochemistry, University of Bath, BATH BA2 7AY, U.K.

The ligands [^{125}I]αbungarotoxin and [^{3}H]nicotine have been used to label putative nicotinic acetylcholine receptors in mammalian brain. Recent evidence indicates that these radioligands label separate binding sites which display marked differences in distribution in the brain; their functional significance is controversial. We have previously undertaken a developmental study of αbungarotoxin in binding to human foetal brain (1), and binding sites for [^{3}H]nicotine in this tissue are now being characterised. High affinity binding sites for [^{3}H]nicotine are present as early as 12 weeks gestation. Numbers of binding sites (40 fmol/mg protein) are comparable with levels found in adult rat brain. However binding sites in P_2 membrane preparations from foetal tissue are much more labile to storage at 4°C, although activity is preserved by freezing the membrane fraction. The presence of receptor sites for nicotine in the foetal CNS has important implications for the adverse effects of maternal smoking on foetal development.

This study was supported by a grant from The Mental Health Foundation.

(1) Whyte, J., Harrison, R., Lunt, G.G. & Wonnacott, S. (1985) Neurochem. Int. 7, 515-523.

DISTRIBUTION OF NICOTINIC BINDING SITES IN RAT BRAIN AND HUMAN FRONTAL CORTEX AS VISUALIZED BY AUTORADIOGRAPHY

A. Adem, A. Nordberg, A. Härfstrand and K. Fuxe
Department of Pharmacology, Univ. of Uppsala and department of Histology, Karoliniska Institute, Sweden.

^{3}H-acetylcholine (^{3}H-ACh) and ^{3}H-nicotine (^{3}H-NIC) bind to sites in brain that have characteristics of nicotinic cholinergic receptors. In the present investigation in vitro autoradiography was used to visualize ^{3}H-NIC and ^{3}H-ACh binding sites in the rat brain. Autoradiography of ^{3}H-NIC (40nM, spec.act. 71.9 Ci/mmol, NEN, USA) binding sites in the coronal section (14 um) of the rat telencephalon at Bregma level +1.2 mm and rat diencephalon at Bregma level -2.3 mm was performed with tritium sensitive film (^{3}H-ultrafilm, LKB, Sweden). The incubation time was 10 min at 4 oC. A 100 uM nicotine solution in Tris-HCl buffer(0.05M, pH=7.5) containing ^{3}H-NIC was used to def unspecific binding. Nicotinic binding sites in human frontal cortex were also assessed. Autoradiography of ^{3}H-ACh (100nM, spec,act. 86 Ci/mmol,Amersham, UK) binding sites was performed in rat diencephalon as described above. The experiments were performed using 50mM Tris buffer(pH=7.4) containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $mgCl_2$ and 1.5 mM atropine sulphate. The sections were preincubated for 10min in Tris buffer followed by 10 min incubation in the buffer in the presence of paraoxon(5mM). The incubation time was 40 min at 4 oC. Our results indicate difference in the distribution of nicotinic binding sites in the different areas of the rat brain with high densities in the thalamus followed next by the cortex. In the thalamus, the highest density was observed in the laterodorsal thalamic nucleus whereas in the cortex, the the frontoparietal area(layer IV) showed highest density of nicotinic binding sites. Preliminary data also show high density of nicotinic binding sites in human frontal cortex. The distribution of nicotinic ^{3}H-ACh binding sites mimics closely that of ^{3}H-NIC binding sites in the rat brain confirming previous results from binding studies using brain homogenates.

BODY TEMPERATURE, BRAIN OXYGEN CONSUMPTION AND CREATINE PHOSPHATE CONTENT IN RATS TREATED WITH ARMIN, DIFAZIN, PAM-Cl AND MINA

R.C. Jović, N.R. Jović.
Belgrade, Vojvode Stepe 280., Yugoslavia.

Rats injected subcutaneously with sublethal doses of ethyl-etoxy--phosphoryl-p-nitrofenolate (Armin) showed a fall of body temperature of 2.0 to 5.0°C in 5 hours, followed by recovery in 10 to 16 hours. At the same time Armin produced a fall in the creatine phosphate content in the central nervous system of up to 60% of control values, a change in sodium and potassium values and a strong inhibition of cholinesterase activity in the blood and cortical slices of the brain. On the contrary, lethal doses of armin produced hypothermia in some animals followed by severe convulsions and exitus. During hypothermia the body temperature was highly dependent on environmental temperature.

Difazin, a central cholinolytic, partly reduced the fall of temperature and the content of creatine phosphate caused by Armin. In this respect PAM-Cl and MINA were ineffective.

It is concluded that Armin causes a temporary disturbance of thermoregulation, probably by interfering with metabolic processes in the central nervous system.

PHARMACOKINETICS AND DISTRIBUTION OF OBIDOXIME-CHLORIDE IN ORGANOPHOSPHATE INTOXICATION

C.Streichenberg, P.G.Waser
Institute of Pharmacology, University of Zurich, Gloriastrasse 32, CH-8006 Zurich, Switzerland

The distribution and kinetics of ^{14}C-labelled Obidoxime-Chloride (Bis-[-4-hydroxy-iminomethyl-pyridinium-(1)-methyl-]-ether, Toxogonin®) were studied by whole-body-autoradiography in male mice. Time intervals between intravenous administration of ^{14}C-Obidoxime-Chloride (0.1 mg/g) and the death of the animal ranged from 2 min to 30 min. In further experiments animals were exposed to Sarin (1.5 µg/g i.v.) and the therapeutic effect of ^{14}C-Obidoxime-Chloride was tested. - The distribution of ^{14}C-Obidoxime-Chloride occured early in the whole body. Blood, liver, kidneys, bladder and organ systems containing cartilage were the concerned organs. The immediate accumulation in the cartilage point to the affinity of quaternary ammonia groups to the SO_4-containing mucopolysaccharides. The continuous decrease of radioactivity in blood and the increase in the kidneys suggest renal excretion of the polar compound. The slow increase of radioactivity in the liver hint to biliary excretion. Whereas mice exposed to Sarin before ^{14}C-Obidoxime-Chloride-application showed higher amounts of radioactivity in blood and lungs; but smaller amounts, increasing with time , in the kidneys and the liver compared to the control animals. Thus, Sarin causes a retardation of elimination. - The CNS (brain and spinal cord) was free of radioactivity in all experiments, therefore Sarin has no influence on the blood-brain-barrier-properties for ^{14}C-Obidoxime-Chloride. - These results indicate that the small therapeutic effect of Obidoxime-Chloride is mainly induced by formation of a complex between Obidoxime-Chloride and Sarin which might be eliminated by the bile or the urine.

CHANGES OF ACETYLCHOLINESTERASE ACTIVITY IN DIFFERENT PARTS OF THE RABBIT BRAIN FOLLOWING DIFFERENT TYPES OF IRRADIATION (UNIFORM AND NON-UNIFORM)

F. Skopec, J. Bajgar
Purkyně Medical Research Institute, 502 60 Hradec Králové, Czechoslowakia

Acetylcholinesterase (AChE, EC 3.1.1.7) activity in the frontal and temporal cortex, mesencephalon, pons, medulla oblongata and cerebellum following irradiation (^{60}Co, 0,4 Gy/min) was studied. The irradiation of the whole body (WB), for the bone marrow (BM), for the abdomen (A) and the head (H) was applied. The activity from the 4-th to 60-th days after irradiation was determined.

AChE activity following WB irradiation was increased (150-200%); most marked changes 22 days after irradiation were demonstrated. On the other hand, diminished AChE activity (60-70%) in the group BM was observed, with most marked changes 29-th day following irradiation. No significant changes were demonstrated if the A irradiation was applied. H irradiation caused an increase of AChE activity (110-120%) with the maximal changes 15 days after irradiation. Some differences of AChE activity in different parts of the brain were also observed.

In general, WB and H irradiation showed similar trend in AChE activity changes differing quantitatively only. BM and A irradiation had either no effect or caused decrease of AChE activity.

It can be concluded that different types of non-uniform irradiation caused an unbalance of AChE in the cholinergic nervous system.

CHOLINESTERASE ACTIVITY AND ITS DIAGNOSTIC VALUE AT DIFFERENT PATHOLOGICAL STATES

J. Bajgar, F. Skopec
Purkyně Medical Research Institute, 502 60 Hradec Králové, Czechoslowakia

Cholinesterases belong to the group of enzymes with large variety of their importance. They are distinguished into two classes - acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BuChE, EC3.1.1.8) which differ in their properties and functions. In the paper presented, we have tried to describe some factors influencing these activities and their diagnostic value. There exist many factors of physical, chemical, physiological and pathological nature influencing both AChE and BuChE activities. Nevertheless, there are some factors of great practical importance - i.e. organophosphates and carbamates, liver diseases, genetical disposition, immunological diseases (ABO incompatibility), Hirschsprung's disease and neural tube defects. The second group of factors is represented by results which are not definitive but they indicate some changes of their activity during malignant tumors, psychiatric disorders, following administration of different drugs, irradiation etc. The third group involves factors giving informations which will require further studies, i.e. connections of cholinesterases with other systems, physiological importance of BuChE, non-cholinergic role of AChE etc. The problems described are discussed.

IMMUNOAFFINITY PURIFICATION AND QUANTITATIVE IMMUNOASSAY OF HUMAN BRAIN ACETYLCHOLINESTERASE.

Z. Rakonczay, P. Hammond and S. Brimijoin.
Department of Pharmacology, Mayo Clinic, Rochester, Minnesota 55905, USA.

A salt-sensitive monoclonal antibody (MAB) to rabbit brain acetylcholinesterase (AChE) (1), with cross-reactivity for human AChE, was conjugated to CNBr-activated Sepharose 4B as an affinity column. Extracts of human cerebellum obtained at autopsy (6-12 hr postmortem) were prepared by homogenization in 50 mM phosphate buffer, pH 7.4, centrifugation at 100,000 g for 1 hr, and re-extraction of the pellet with buffer containing 0.5% Triton X-100. The extracts were applied to the affinity column, which was rinsed and eluted with 2 M NaCl (61.8% yield, 150-fold enrichment). Peak fractions of AChE were further purified by affinity chromatography with phenyltrimethylammonium Sepharose (final specific activity 1254 IU/mg protein). Crude and purified human brain AChE were then used to validate a new two-site radioimmunoassay. The antigen was exposed to an immobilized MAB in microtiter wells for 2 hr at 23°. After rinsing, ^{125}I-labeled MAB directed toward a non-overlapping epitope was added. Specifically bound radioactivity was a linear function of the amount of enzyme added over the range, 0.2-20 ng, with no difference between crude or purified samples. This sensitive assay may be useful in studies on the content and regulation of AChE in neural tissue. (Supported in part by grant NS 11855, U.S. Public Health Service).

(1) K.P. Mintz, S. Brimijoin (1985) J. Neurochem. 44:225-232.

EFFECTS OF MUSCULAR FATIGUE ON ACTIVITIES OF CHOLINE ACETYLTRANSFERASE (ChAT) AND ACETYLCHOLINESTERASE (AChE) IN RAT SKELETAL MUSCLE

B. Birner, U. Heyme, G. Feustel
Department of Biochemistry, Research Institute of Physical Culture and Sports, Leipzig, GDR

Muscular fatigue was induced by electric stimulation (indirectly via the nerve) of the m. extensor digitorum longus using quadrouplet square wave pulses. The process of muscular fatigue was followed up by a simultaneous isometric measurement of the muscular strength. Complete muscular fatigue was assumed to be reached if the muscular strength was reduced up to 10 % of the starting value.

The activities of ChAT and AChE which were determined at several stages of the muscular fatigue do not reflect the decrease of muscular strength. Rather muscular fatigue was accompanied by an alteration of the pattern of AChE molecular forms. This result indicates the possibility of a complex interrelation of the regulatory function of certain molecular forms of AChE.

POSTERS - SECTION C

CATECHOLAMINES AND SEROTONIN

THE METABOLISM OF ^{3}H-ADRENALINE (AD) IN THE RAT BRAIN

G. Köster, Institut für Klinische Biochemie, Neurochirurgische Universitätsklinik, Bonn, F.R.Germany.

The metabolism of intraventricularly injected DL-(7-^{3}H)AD and DL-(7-^{3}H)noradrenaline (NA) was studied in several rat brain areas. Less ^{3}H-AD than ^{3}H-NA was recovered in hypothalamus (HT) and striatum (ST), but in pons+medulla (PM) more ^{3}H-AD than ^{3}H-NA was found. Almost similar amounts of total ^{3}H-metabolites and of methylated products were formed from the two amines, but ^{3}H-AD and ^{3}H-metanephrine were deaminated less effectively than ^{3}H-NA and ^{3}H-normetanephrine, respectively. Reserpine, when given 1h after the amines, reduced ^{3}H-AD more than ^{3}H-NA in HT, but in PM it was less effective. A compensating increase of total ^{3}H-metabolites was seen only in HT of ^{3}H-AD treated rats.

The results show that ^{3}H-AD has access to all the enzymes of the NA metabolism, but has a lower affinity for MAO than NA. Compared to ^{3}H-NA the neuronal storage of ^{3}H-AD was reduced (except in PM which contains adrenergic nerve cells), and its extraneuronal metabolism was shifted from a site of slow deamination to another with a faster deamination.

^{3}H ISOTOPE EFFECTS ON THE METABOLISM OF ADRENALINE (AD) IN RAT BRAIN

G. Köster, Institut für Klinische Biochemie, Neurochirurgische Universitätsklinik, Bonn, F.R.Germany.

The metabolite patterns of L-(N-methyl-2,5,6-^{3}H)AD ((Me-^{3}H)AD) were determined in several rat brain areas 10 or 60 min after intraventricular injection. They were approximately corrected for the loss of methyl ^{3}H and compared to the patterns of DL-(7-^{3}H)AD. Less (Me-^{3}H)AD than (7-^{3}H)AD was recovered. The same total amounts of methylated metabolites were found in the two groups, but (Me-^{3}H)AD and (Me-^{3}H)metanephrine both were deaminated slower than the corresponding (7-^{3}H)compounds. Some final extraneuronal metabolites seemed to disappear faster when formed from (Me-^{3}H)AD.

The marked retardation of the deamination is probably caused by the N-methyl ^{3}H. It seems that ^{3}H also inhibits the neuronal uptake and shifts the extraneuronal metabolism to a site with a higher clearance rate of final metabolites, but it is not clear at which molecular position it does so.

DISTRIBUTION OF PHENYLETHANOLAMINE-N-METHYLTRANSFERASE IN RAT HEART: EFFECT OF 6-HYDROXYDOPAMINE

T. Torda, J. Čulman, M. Petríková.
Institute of Experimental Endocrinology, CPS SAS, Bratislava, Czechoslovakia.

The activity of epinephrine forming enzyme-phenylethanolamine-N--methyltransferase (PNMT) was measured in the atria and ventricles of rat heart.
The PNMT activity in rat heart is very low, nevertheless significantly higher PNMT activity was found in rat atria compared to the ventricles. In examined parts of rat heart PNMT activity correlate well with the norepinephrine content. Systemic administration of 6-hydroxydopamine (100 mg/kg, 3-times) caused 90% depletion of norepinephrine in all parts of rat heart without any changes of PNMT activity in rat atria. Significant increase of PNMT activity was found in the ventricles of rat heart in 6-hydroxydopamine treated rats compared to saline treated controls.
These results may suggest that epinephrine forming enzyme- PNMT in rat heart is localized extraneuronally or postsynaptically.

TISSUE LEVELS OF NEUROTRANSMITTER AMINES AND THEIR METABOLITES IN A FELINE BRAIN TUMOR MODEL

J. Bayens-Simmonds, D.P.J. Boisvert, and G.B. Baker*.
Department of Surgery and Psychiatry*, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2G3.

Alterations in brain catecholamines and 5-hydroxytryptamine (5-HT) have not been studied extensively in peritumor brain edema. Using a feline brain tumor model, we measured levels of noradrenaline (NA), dopamine (DA), 5-HT, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindole-3-acetic acid (5-HIAA) and percent water in tumor and peritumor and remote gray and white matter.

1 x 10^6 rat 9L gliosarcoma cells were xenotransplanted into the central white matter of the right hemisphere. Fourteen days later the brains were frozen in vivo with liquid N_2. The brains were sliced coronally through the tumor and samples of tumor, peritumor and remote white and gray matter and tissues from equivalent areas in the contralateral hemisphere were dissected out. Amines and metabolites were analyzed by high pressure liquid chromatography with a reversed phase C_{18} column and electrochemical detector and water content was measured by oven drying.

Neurotransmitter amines and their metabolites were very low in the 9L glioma tumor and DA and its metabolite HVA were significantly decreased in gray matter adjacent to the tumor. Since water content was not increased in gray matter, the decrease in neurotransmitter level was not a dilution effect of the edema. Decreased cortical function observed in brain tumor patients may be related to impaired neurotransmitter metabolism.

Funds were provided by the Alberta Cancer Board and Alberta Heritage Foundation for Medical Research.

DETECTION OF HAMSTER TYROSINASE BY RADIOIMMUNOASSAY

J. Vachtenheim, J. Ruchoň.
Department of Biochemistry, Faculty of General Medicine, Charles University, Prague, Czechoslovakia.

Tyrosinase /EC 1.14.18.1/ is a marker enzyme of pigment cells which originate in the neural crest. Hamster melanoma tyrosinase has been purified to homogeneity as checked by SDS-PAGE and radioiodinated with ^{125}I. Using the labeled enzyme and polyvalent rabbit immunoglobulins to hamster tyrosinase /1,2/, it was possible to quantitate the enzyme by radioimmunoassay. The assay detects a minimum level of 60 ng of tyrosinase per ml of serum or tissue homogenate. The highest concentrations of tyrosinase were found in primary hamster melanoma tissue and in hamster melanotic serum. The enzyme concentration in hamster melanotic serum was found 5 to 15-fold elevated when compared with control hamster serum. The determination of tyrosinase by radioimmunoassay may be of great importance since widespread occurence of tyrosinase inhibitors /3/ hampers the estimation of its enzymatic activity in crude samples.

/1/ J.Vachtenheim et al. /1973/ Physiol. Bohem. 22:405-411.
/2/ J.Vachtenheim et al. /1973/ J. Invest.Derm. in Press.
/3/ T.Horton, H.R.Waterman /1971/ Experientia 27:644-645.

QUANTITATIVE AUTORADIOGRAPHIC DISTRIBUTION OF GLUTAMATE AND ASPARTATE HIGH-AFFINITY UPTAKE SYSTEM IN THE RAT HIPPOCAMPAL FORMATION DURING POSTNATAL DEVELOPMENT

W. Schmidt, G. Wolf.
Institute of Biology, Medical Academy, DDR-3090 Magdeburg, German Democratic Republic

Quantitative autoradiography was used to determine the topographical and temporal distribution of L-[^{3}H] glutamate and D-[^{3}H] aspartate high affinity (HA) uptake system in the hippocampal formation of the rat during postnatal development. In control experiments autoradiograms of hippocampal slices incubated at low temperature (0 to 4°C) or omitting sodium ions were devoid of radioactivity. The glutamate and aspartate HA uptake increased markedly during the second week of postnatal development in hippocampal dendritic layers. The method used did not show any significant labeling of cell body layers (stratum pyramidale and granulosum) of young and adult rats. Dendritic layers from animals prior to postnatal day 10 accumulated D-[^{3}H]aspartate and L-[^{3}H]glutamate poorly as well. Our data suggest that the HA uptake system is expressed in glutamatergic and aspartatergic structures of hippocampal formation only when they are in an advanced state of differentiation.

DEVELOPMENT OF GLUTAMATE DEHYDROGENASE ACTIVITY IN MATURING GLUTAMATERGIC STRUCTURES OF THE RAT CEREBELLUM

G. Schünzel, G. Wolf
Institut of Biology, Medical Academy, DDR-3090 Magdeburg, German Democratic Republic

Several lines of evidence suggest that the granule cells of the cerebellar cortex are glutamatergic (1). At present is not clear which enzymes are involved in the metabolism of transmitter glutamate (2). Accordingly, the development of the activity of glutamate dehydrogenase (GDH) was determined by quantitative histochemistry in the rat cerebellum during postnatal maturation. The activity of the enzyme increased markedly in the cerebellar cortex during the first weeks of life. On day 40, the distribution pattern observed was similar to that in adult animals. Whilest the external granular layer showed only low GDH activity, by the way of migration of granule cell perikarya inwards to the molecular and internal granular layer the GDH activity increased 4fold, the enzyme activity of molecular neuropil enhanced about 3fold. The findings suggest that GDH plays a role in the synthesis and/or regulation of transmitter glutamate.

(1) M. Ito (1984) Biomed. Res. 5, Suppl.: 63-66
(2) J. Drejer et al. (1985) Neurochem. Res. 10:49-62

D 20, D 21

KAINIC ACID SEIZURES, AMINE TURNOVER, AND HISTOPATHOLOGY

M.L.Berger, H.Lassmann & O.Hornykiewicz.
Institute of Biochemical Pharmacology, Univ.Vienna, Borschkegasse 8A, A-1090 Vienna, Austria.

Stereotaxic injection of kainic acid into the amygdala of rats induces limbic seizures and, as a consequence, widespread neuronal degeneration, especially in the ipsilateral hippocampal formation. We observed the most pronounced neurochemical changes in the injected amygdala itself and in the CA3-part of the ipsilateral hippocampus. Five hours after injection of 0.8 µg kainic acid, noradrenaline levels were reduced to 25% of control level in the CA3 region, and serotonin turnover was doubled. Very similar changes in these amines were seen after intraamygdaloid injection of the non-convulsive neurotoxin ibotenic acid, which did not induce any neuronal damage in CA3. It is concluded, that there is no direct relationship between the dramatic increases in noradrenergic and serotonergic activity during kainic acid-induced seizures and the histopathological changes.

This study was supported by the Austrian Science Research Fund, project number P3647.

AUTORADIOGRAPHY OF ^{3}H-D-ASPARTATE IN THE RAT OLFACTORY BULB

N.Halász
Inst. Biophysics, Biol.Res.Ctr. of the Hungarian Academy of Sciences, 6701 Szeged, P.O.Box 521

During the former decade, attempts were made, among others, also in our laboratory to localize several neurotransmitters in the olfactory bulb, through the autoradiographic analysis of the uptake of labeled mediator substances or their precursors. Based to these experiments, we contributed to the better knowledge of the localization of dopaminergic /1-4/, noradrenergic /3,4/ and GABA-ergic /5,6/ neuronal sites. We were, however, unable to detect sites for aspartate, or glutamate uptake until yet, although the olfactory bulb was found to be very rich in these two amino acids /7/. By the use of labeled D-aspartic acid, presumed aspartate-ergic structures were revealed mostly in the granule cell layer, according to the present study.

/1/ Hökfelt et al./1975/ Neurosci.Lett.1:85-90. /2/Halász et al. /1977/ Brain Res. 126:455-474. /3/Halász et al./1978/ Brain Res. 154:253-271. /4/Halász et al./1983/ Neuroscience 8:705-715. /5/Halász et al./1979/ Brain Res. 167:221-240. /6/Halász et al. /1981/ Neuroscience 6:473-479. /7/Popov et al./1967/ Acta biol.med.ger. 18:695-702.

QUANTITATIVE AUTORADIOGRAPHIC CHARACTERIZATION OF Cl^- INDEPENDENT GLUTAMATE BINDING SITES IN MOUSE BRAIN

A. Mitsacos, F. Angelatou, E.D. Kouvelas.
Department of Physiology, Medical School, University of Patras, Greece.

In vitro autoradiography was used to characterize and quantitavely map the binding of ^{3}H-L-glutamate in mouse brain. We have used the cerebellum as a model system to investigate possible subpopulations of glutamate binding sites. Glutamate binding to frozen cerebellar sections was measured in the absence of chloride, calcium or sodium ions. Scatchard analysis of glutamate binding by liquid scintillation counting suggested a single population of binding sites with a Kd of about 300nM and a maximun number of sites of 3.16 pmole/mg prot. Quantitative densitometry indicated similar dissociation constants for the granule cell and the molecular layer of the cerebellum. However, a higher concentration (almost double) of L-glutamate binding sites was observed in the granule cell than in the molecular layer of the cerebellum. The pharmacological characteristics of glutamate binding were assesed in cerebbelar sections and layers, by measuring the displacement potency of L-glutamate, L-aspartate, NMDA, ibotenic, quisqualic and kainic acid. It appeared that the granule cell layer displayed a pharmacological profile which differed from that of the molecular layer. The distribution of the Cl^- independent glutamate binding sites in the mouse CNS was also measured by quantitative densitometry, taking into account the different quenching between brain areas due to the autoabsorption of tritium (1). The distribution correlated well with structures which are viewed as major glutamatergic targets, such as striatum, cortex, hippocampus and cerebellum.

(1) W.A. Geary, G.F. Wooten (1985) Br. Res. 336, 334-336

L-GLUTAMATE RECEPTORS IN THE CEREBELLUM AND ELECTRIC LOBE OF TORPEDO MARMORATA

A. Mitsacos[1], P. Giompres[2]
Dept. of Physiology, Medical School(1), Dept. of Human and Animal Physiology(2), University of Patras, Greece.*

Only limited information is available about glutamate receptors in lower vertebrates. In the CNS of the elasmobranch fish Torpedo marmorata, which possesses bilateral electric organs, the distribution of free amino acids has shown a rather high concentration of glutamate in all areas(1). We have examined the ^{3}H-L-glutamate binding to frozen brain sections of Torpedo marmorata, in the absence of chloride, calciun and sodium ions. In the cerebellum and electric lobe, the glutamate binding was specific, saturable and reversible with a Kd of about 265nM, indicating a similar affinity to the mammalian glutamate receptor. The maximum number of binding sites was three times higher in the cerebellum (Bmax=78.78nmol/mg prot.) than in the electric lobe (Bmax=25.00nmol/mg prot.). Quantitative autoradiography indicated that the highest concentration of glutamate binding sites among Torpedo brain regions, was in the molecular layer of the cerebellum. The granule cell layer showed about four times less glutamate binding sites. All other regions, including the electric lobe, exhibited moderate levels of glutamate binding, which are however much higher than the levels of glutamate binding in mammals. The pharmacological specificity of the glutamate binding sites was also studied in the cerebellum and electric lobe. Glutamate and quisqualate were potent displacers of the Cl^-/Ca^{2+} independent glutamate binding, while NMDA exhibited a 40% maximum inhibition. This study supports the role of glutamate as a neurotransmitter of the cerebellum and could be used as a first implication of a similar role of glutamate in the electric lobe of Torpedo marmorata.

(1) L.P. Davies, G.H.C. Dowe (1979) Comp. Biochem. Physiol. 62C, 107-113.

* A major part of this work was done in the Abt. fur Neurochemie, MPI, Gottingen, FRG (director V.P. Whittaker).

K 14, K 15

SOLUBILIZATION AND CHARACTERIZATION OF THE APAMIN RECEPTOR ASSOCIATED WITH A Ca^{2+} ACTIVATED K^+ CHANNEL FROM RAT BRAIN.

B. Marquèze, M.J. Seagar and F. Couraud.
Laboratoire de Biochimie, Faculté de Médecine Nord, Bd. P. Dramard F- 13326 Marseille Cedex 15. France.

Apamin, a peptide neurotoxin from bee venom, blocks the Ca^{2+} activated K^+ current responsible for the slow hyperpolarization which follows the action potential of certain neurons. This K^+ conductance seems to play an important role in regulating repetitive activity in excitable cells. Mono ^{125}I iodoapamin binds with high affinity to intact cultured neurons (Kd = 90 pM, Bmax = 500 - 1000 sites/cell) and synaptic membranes (Kd = 30 pM, Bmax = 30 *fmol/mg* protein). Photoaffinity labeling with arylazide ^{125}I-apamin derivatives has shown the presence of 86, 59, 33 and 22 *kDa* membrane polypeptides in the environment of receptor site. The apamin binding protein was solubilized from rat brain synaptic membranes using sodium cholate. The receptor retained high affinity for mono iodoapamin with Kd = 40 pM at pH 7.5 and 1°C and a binding capacity of 17 fmol/mg protein. Apamin binding was stimulated by K^+ with a $K_{0.5}$ = 0.6 mM as in synaptic membrane, demonstrating that the regulatory K^+ site is also part of the soluble complex. Analysis of the covalently labeled apamin binding protein/sodium cholate complex by density gradient centrifugation indicated a high molecular weight with $S_{20,w}$ = 20 S.

PROPERTIES OF La^{3+}-INDUCED $^{45}Ca^{2+}$ UPTAKE IN HUMAN RED BLOOD CELLS

Ľudovít Varečka, Elena Peterajová, and Jozef Pogády

Mental Health Research Center of the Institute of Medical Bionics, Psychiatric Hospital, 902 18 - Pezinok, Czechoslovakia

The uptake of $^{45}Ca^{2+}$ induced by La^{3+} ions /1/ was studied in human red blood cells (RBC) of healthy volunteers. The increase of extracellular K^+ concentration from 5 to 70 mmol/l stimulated the uptake up to 4 times. The dependence of the extent of the $^{45}Ca^{2+}$ uptake stimulation on the extracellular K^+ concentration was approx. linear in a semi-logarithmic scale. Choline chloride, when used instead of KCl, was ineffective in the $^{45}Ca^{2+}$ uptake stimulation. On the other hand, the substitution of remaining Na^+ in the 70 mmol/l K^+ medium for choline led to the reduction of the $^{45}Ca^{2+}$ uptake. The stimulation by high-K^+ of the La^{3+}-induced $^{45}Ca^{2+}$ uptake was more expressed in heparinized blood as compared with chelatonate-treated blood. In the latter, the inhibition of the $^{45}Ca^{2+}$ uptake was often observed instead of its stimulation. The results show that the depolarization-induced inward Ca^{2+} transport is present in the RBC membrane.

/1/ Szász,I.,Sarkádi,B.,Schubert,A.,and Gádros,G. (1978) Biochim. Biophys.Acta 512, 331-340

DISTRIBUTION OF FAST AXONAL TRANSPORT IN REPAIRED NERVES

M. Frizell, N. Danielsen
Departments of Neurology and Anatomy, Sahlgren Hospital, S-413 45 Göteborg, Sweden

We used the distribution of fast anterograde phase of radiolabelled axonal proteins (1) to follow the regeneration of transected rabbit hypoglossal nerves in comparison to nerve crush. The nerves were repaired with either mesothelial chamber (2) or epineurial suture, 5-90 days before radiolabelling. Outgrowth delay was 4.8 and 5.7 days for suture and chamber reconnected nerves respectively and 2.5 days after nerve crush. Regeneration was also verified by light-microscopy and electro-myographic recording. The regeneration rate was 2 mm/day in either type of repair as compared to 4 mm/day after crush. Total incorporation of radioactivity was greater in the regenerating nerves as compared to contralateral nerves in either type of repair at all time intervals. Axonal transport was not normalized even after 90 days after repair. A major finding was a persisting peak of accumulated radioactivity at the site of lesion in repaired nerves which may indicate persisting neuroma formation at the scar. The peak contained 60% of the total radioactivity of the nerve after 7 days and 40% after 90 days. Crushed nerves in contrast only showed a slight transient peak of radioactivity at site of lesion during the first two weeks. Pattern of axonal transport showed no significant difference between the two techniques of repair, whereas they differed markedly as to crushed nerves.

(1) M. Frizell, J. Sjöstrand (1981) in Traumatic Peripheral Nerve Regeneration (A. Gorio et al.eds.) pp 35-44, Raven Press, New York.
(2) G. Lundberg et al. (1982) J. Hand Surg. 7:580-587.

FORMATION OF A HIGHLY VISCOUS NETWORK IN VITRO BY PURIFIED NEUROFILAMENTS (NF) ALONE. PHYSICOCHEMICAL PROPERTIES AND SPATIAL ORGANIZATION

J. EYER, J.F. LETERRIER. Centre de Neurochimie, Strasbourg, France

The unique composition and structure of NF is supposed to be involved in the heavy cross-bridging described in situ in axonal NF bundles and between NF and other subcellular organelles. The direct participation of the 200 kD subunit in the interfilament reticulation was recently demonstrated (1). These findings suggest that NF can establish direct physical links with other structures. This hypothesis is supported by the present results : freshly purified NF from spinal cord form spontaneously a highly viscous gel in vitro. The extent and velocity of the viscosity change depends on temperature, protein concentration (with a critical concentration of 1.5 mg/ml) and is fully reversible after gentle shearing of the gelled sample. The phenomenon is specific of NF (no viscosity change with purified GF preparation), highly sensitive to proteolysis and denaturing conditions (lack of recovery of the gel forming property of NF solubilized in 8 M urea and reconstituted by dialysis). With a slightly acidic optimum pH (6.5-6.8), the reaction is activated by Mg^{++} (in a mM concentration range) and ATP between 0.1-0.5 mM. However, higher concentration of ATP (2-5 mM) and the high phosphorylation level of NF proteins were found to inhibit strongly the formation of the gel. This effect is reversed by addition of phosphatase to the sample. Several proteins (MAPs, denatured NF, BSA) decrease the viscosity rate of native filaments, which is activated by a fraction of soluble proteins associated with NF during the first step of their purification. These results, together with the morphological observations of the gelled sample which demonstrate the in vitro formation of very long birefringent bundles of NF interlinked through cross-bridges, argue for a specific interaction between NF, possibly mediated by their HMW subunits, and modulated by their phosphorylation. Such a model in vitro could reflect some of the dynamic properties of the NF in the axoskeleton network.

(1) Hirokawa N. et al. (1984) J. Cell Biol., 98, 1523-1530.

METABOLIC MAPPING OF THE CEREBRAL EFFECTS OF BENZODIAZEPINES IN RATS.

M. Dam, G. Pizzolato, C. Ori, U. Freo, L. Battistin.
Department of Neurology, University of Padua, Italy.

The (^{14}C)deoxyglucose method is an autoradiographic technique to measure local cerebral glucose utlization (LCGU) throughout the CNS (1). This method has been used to map the modifications of brain functional activity in response to various drugs.

We used this technique to investigate the time-course and the regional distribution of the cerebral metabolic effects of lorazepam and flunitrazepam in relation to their behavioral effects in the rat. Benzodiazepines decreased LCGU in about 60% of the regions examined. The topographical distribution os the affected regions closely correspond to the known distributions of the (^{3}H)flunitrazepam binding sites (2). LCGU was significantly reduced in many neocortical areas, in the amygdaloid complex, in the hipppocampus, in the reticular formation, and in the cerebellar cortex.

The time-course of the metabolic changes after benzodiazepines correlates with duration of their behavioral effects and with the occupancy of specific binding sites (3).

(1) Sokoloff L. et al., Neurochem. 28,897,1977.
(2) Young W.S. and Kuhar M.J., J. Pharmacol. Exp. Ther. 212,337,1980.
(3) Jack M.L. et al., Prog. Neuro-psychopharmacol. Biol. Psychat. 7,629,1983.

EFFECTS OF CHRONIC ANTIDEPRESSANT TREATMENT ON TISSUE LEVELS, BINDING PROPERTIES AND IN VITRO RELEASE OF SUBSTANCE P IN THE RAT SPINAL CORD

K. Iverfeldt, L.-L. Peterson, S.-O. Ögren[X], E. Brodin[XX], T. Bartfai
Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, Stockholm [X]R & D Laboratories Pharmacology, Astra Läkemedel AB, Södertälje and [XX]Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

Chronic treatment with zimelidine, a selective serotonin (5-HT) uptake inhibitor, (2 x 10 µmol/kg/day p.o. for 14 days) resulted in lowered tissue levels of the 5-HT metabolite; 5-hydroxyindol acetic acid (5-HIAA) and elevated tissue levels of substance P (SP) in both the ventral and dorsal spinal cord. The increase in SP levels was more pronounced in the ventral spinal cord, where most of the SP is in serotoninergic neurons, than in the dorsal spinal cord. Chronic treatment with imipramine (2 x 10 µmol/kg/day p.o. for 14 days) resulted in qualitatively similar, but less pronounced, changes of the tissue levels. The amount of SP released (pmol/g tissue) and the fractional SP release upon stimulation by K^+(40 mmol/l) from slices of the ventral spinal cord from chronically treated rats were increased after zimelidine but not after imipramine treatment. Substance P receptors were examined in crude synaptosomal fraction preparations of spinal cord from chronically treated rats using ^{125}I-Bolton Hunter substance P(^{125}I-BHSP). Chronic treatment with imipramine caused a significant reduction of the number of substance P binding sites in both the dorsal and ventral spinal cord.

CONJUGATED CATECHOLAMINES IN CEREBROSPINAL FLUID OF PATIENTS WITH PARKINSON'S DISEASE.

T.Ohuchi, Y.Ishimura, T.Tsunematsu, M.Oka, Y.Nakagawa* and K.Matsumoto*.
Department of Pharmacology, and *Department of Neurosurgery, Tokushima University School of Medicine, Kuramoto-3, Tokushima 770, Japan.

Present study deals with the measurement of free and conjugated catecholamines(CAs)(1) and their metabolites, VMA and HVA, in cerebrospinal fluid(CSF) of patients with Parkinson's disease, as indices of the rate of CA utilization in the brain in this disease. Conjugates of CAs in human CSF were mostly NA-and DA sulfates(2). Mean DA sulfate in CSF was elevated in Parkinsonian patients who were confirmed to have not been treated with L-DOPA for at least 3 months [843.4±210.2(S.E.)pg/ml vs control 302.6±28.5(S.E.)pg/ml]. However NA sulfate, VMA, HVA, free DA nad NA in CSF of the patients were not significantly altered.
Although there is a marked deficiency of striatal DA in the brain of patients with Parkinson's disease, our results suggest that the patients are still able to synthesize a considerable amount of DA, but only a small portion of DA produced may be stored in the neurons, the rest may be conjugated in the tissues and flow out into CSF.

(1) M.Da Prada and G.Zürcher : Life Sci., 19 : 1161-1174(1976)
(2) T.Tsunematsu : Tokushima J. exp. Med., 32 : 23-29(1985)

KAINATE AFFECTED CAUDATE NUCLEUS IN THE CAT IMAGED IN VIVO BY POSITRON EMISSION TOMOGRAPHY (PET) OF $^{55}CoCl_2$.

J.B.P. Gramsbergen*, J. Korf*, A. Paans** and W. Vaalburg**
*)Department of Biological Psychiatry and **)Department of Nuclear Medicine, University Hospital, Oostersingel 59, 9713 EZ Groningen, The Netherlands.

Excitotoxic lesions of the rat striatum mimic some neurochemical changes in Huntington's disease (1) and calcification of the basal ganglia in Fahr's disease (2).

It was shown that endogenous calcium (Ca) and intravenously injected $^{45}Ca^{2+}$ accumulate in kainate affected brain tissue. Ca has no positron emitting isotope usefull for application in PET. In the rat radioactive cobalt (Co) and strontium (Sr) - both divalent cations - accumulate in brain areas damaged by kainate, as does ^{45}Ca. Sr is not usefull for PET of the brain, because it accumulates predominantly in the bone.

Using PET, we were able to visualize lesions of the cat caudate nucleus, produced by 0.5 and 1.0 ug kainate, approximately 24 hours after the intravenous administration of 100 uCi $^{55}CoCl_2$. The half-life of ^{55}Co is 17 hours 50'. These lesions were not detectable by X-ray CT.

In addition to neurotoxic lesions, the usefullness of $^{55}Co^{2+}$ to monitor brain damage by PET will be evaluated in other types of lesions, such as caused by hypoxia or mechanical intervention.

This study is supported by the Dutch Organization for Fundamental Medical Research, FUNGO (grant 900-552-048).

References
1. Coyle JT and Schwartz R (1976), Nature 263: 244-246
2. Korf J and Postema F (1984), J.Neurochem 43: 1052-1060.

ENZYMOLOGY OF DNA REPLICATION AND REPAIR IN THE BRAIN

C.C. Kuenzle
Institut für Pharmakologie und Biochemie, Universität Zürich-Irchel, CH-8057 Zürich, Switzerland

A number of enzymes thought to be involved in DNA replication have been identified in brain. These include single-stranded DNA-binding proteins, topoisomerases I and II, DNA polymerase α, a protein that binds Ap_4A and might be classified as a DNA polymerase α accessory protein, RNase H, DNA polymerase β, DNA ligase, an endo- and an exonuclease of unknown function, DNA methyl transferase and poly(ADPR) synthase.

In contrast, little is known about the enzymology of DNA repair in brain. The few enzymes identified comprise uracil-DNA glycosylase, DNA polymerase β, DNA polymerase α (which in neurons is present only at immature stages), DNA ligase, poly(ADPR) synthase, and O^6-alkylguanine-DNA alkyltransferase. In addition, an exonuclease acting on depurinated single-stranded DNA (tentatively listed here as 3' -> 5' exonuclease), an endonuclease of unknown function as well as ill-defined acid and alkaline deoxyribonucleases also occur in brain.

SUBCELLULAR FRACTIONATION OF BRAIN METABOLIC DNA

T. Menna, F. Morelli, C. Buono and A. Giuditta[1]
International Institute of Genetics and Biophysics, Via Marconi 10, Naples 80134, Italy. [1]Dept.Gen.Environ.Physiol., Univ.Naples.

Brain DNA undergoes a process of turnover which is modulated by learning and sleep (1). To examine the subcellular distribution of newly-synthesized brain DNA, adult rats received 100 µCi [3H-methyl] thymidine (intraventricularly: 40 µCi/mmol; Amersham International) and were killed after 3 hr. Isotonic sucrose homogenates of the brain were grossly fractionated into nuclear, mitochondrial and microsomal fractions, following routine procedures. Our results indicate that radioactive brain DNA is largely associated with the nuclear fraction, and in minor amounts with the mitochondrial and microsomal fractions. The microsomal fraction seems to be the first one to become labelled, following injection of the radioactive precursor. Radioactive DNA is also present in purified mitochondrial and synaptosomal fractions. Among fractions of pure nuclei, it prevails in fractions of small nuclei, presumably of oligodendroglial origin. Centrifugation of the brain microsomal fraction on sucrose gradients indicates that the pattern of sedimentation of radioactive DNA is partially different from the corresponding patterns obtained with liver or kidney.

(1) A. Giuditta et al. (1986) in "Role of RNA and DNA in Brain Function" (A. Giuditta et al. eds.), pp. 268-279, Martinus Nijhoff Publ., Boston.

DNA REPAIR AND TURNOVER IN NERVE CELLS

T. Ishikawa and T. Kitagawa
Department of Experimental Pathology, and Department of Pathology, Cancer Institute, Tokyo 170.

Relatively little is known about DNA repair and turnover in neuronal cells. We reported autoradiographic procedures for detecting DNA repair in cerebral ganglion cells of aquarium fish *in vivo*(1) and in rat retinal ganglion cells in organ culture(2). By these methods, active unscheduled DNA synthesis(UDS) was demonstrated over the nuclei of ganglion cells after their exposure to a wide variety of chemical carcinogens and UV light. Using a fish brain system, we compared DNA repair in 5 species of fish, and demonstrated UDS in all of them in response to 2 carcinogens, 4HAQO and MMS(3). We conclude that ganglion cells show very active DNA excision repair.

DNA turnover in rat retinal ganglion cells was also investigated(4). Developing retinas of newborn rats were labelled by multiple injections of (methyl-^{3}H)thymidine. Careful autoradiographic studies of the eyes showed no detectable DNA turnover in photoreceptor cells during the experiment(730 days). Thus the DNA of postmitotic ganglion cells, which are not replaced throughout the life span of the animal, is apparently very stable and is possibly protected in some special manner.

(1) T. Ishikawa, et al. (1978) Virchow Arch. B. 28:235-242.
(2) T. Ishikawa, et al. (1978) J. Natl. Cancer Inst. 61:1101-1105.
(3) T. Ishikawa, et al. (1984) Natl. Cancer Inst. Monogr. 65:35-43.
(4) T. Ishikawa (1985) Cell Tissue Res. 232:445-448.

HISTOAUTORADIOGRAPHIC DETECTION of "IN VIVO" COURSING DNA REPAIR SYNTHESIS in DIFFERENT ORGANS of MICE

J. Hochmann
Research Institute for Organic Syntheses Pardubice-Rybitví

We develop the method for histoautoradiographic detection of unscheduled DNA synthesis (UDS) "in vivo" induced by carcinogens.Main difficulty for us is a removal of free or nonspecific bound radioactivity (background).This complication we eliminated partially by prolonging the time between the ^{3}H thymidine (dTh) application and animals killing (1).So using the time interval 7,5 or 24 hours we detected UDS in testicles,pancreas and intestinal peritoneum and smooth musculature after methylnitrosourea (MNU) and in liver,kidney and lung after MNU and dimethylnitrosamine.

In case of some other organs or of only weak DNA damage there rise a necessity to prove some other method of background elimination.This is but time-pretentious,because a labeling of UDS after whole-body application of ^{3}HdTh is poor and for its detection it is necessary to use very long exposition time of autoradiograms.In tissues of a splanchnocranium the exposition time needed is more than one year.For this reason we decided to increase the dose of ^{3}HdTh from 2 to 20 MBq/g.It was not possible to increase a volume of injected solution.Therefore the producer of ^{3}HdTh complied with our wish and elevated for us ^{3}HdTh concentration from usual 40 to 400 MBq/ml.Such elevation of the ^{3}HdTh dose resulted in shortening of autoradiograms exposition time in our orientation experiments.We suppose that this more concentrated ^{3}HdTh solution will be advantageous for the UDS detection using whole-body application of this precursor,not only in case of splanchnocranium,but also in neurocranium.

(1) J. Hochmann (1983) Neoplasma 30/1:13-22.

AUTORADIOGRAPHIC STUDY OF DNA SYNTHESIS IN THE BRAIN OF ADULT FROGS

G. Bernocchi , E. Scherini, V. Mareš*
Dipartimento di Biologia Animale, Centro di Studio per l'Istochimica del C.N.R., Pavia, Italy;*Institute of Physiology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia

Cell proliferation was studied by Kirsche (1) in the frog brain, with reference to the matrix zone. The present study reports more extensive autoradiographic analysis of the proliferation of ventricle and parenchymal brain cells of adult frogs, which were injected with 2.5μCi/g b.w. of ^{3}H-thymidine and killed 2h later. Labelled cells (LCs) are present around the ventricles or the paraventricular areas with variable frequency. In the lateral ventricles of the telencephalon and in the 3rd and 4th ventricles, the labeling index (LI) is about 0.1%, while in the infundibulum and in the recessus preopticus it is 1-2%. In the choroid plexuses LI is 2%. LCs in the parenchyma are rare (LI<0.1%), except for the nucleus of the hippocampal commissure (LI= 3%). LCs in the parenchyma are mainly glial or endothelial; there are labelled microneuron-like cells in the nucleus of the hippocampal commissure. If the data obtained in mouse (2) and frog are compared, it is clear that the distribution of DNA synthesizing or dividing brain cells is rather conservative during phylogenesis, although their relative number is generally lower in the frog. The high frequency of new cells in an olfactory area may be linked to a compensatory stimulus due to the enhancement of the olfactory system during adaptation to the terrestrial environment.

(1) W.Kirsche (1967) Z.mikrosk-anat.Forsch.77: 313-406
(2) V.Mareš (1985) J.Neurogenetics 2: 160-162

ACCURACY OF AUTORADIOGRAPHIC H3-DNA-RETENTION METHOD FOR STUDIES OF CELL FORMATION IN THE DEVELOPING BRAIN

J. Sýkorová, V. Mareš.
Institute of Physiology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia.

H3-thymidine has been widely used for autoradiographic estimation of terminal mitosis time (birth-dates) of brain cells, particularly neurons.

16-day-old embryos (ED 16) and 5-day-old offsprings (PD 5) of Wistar rats received H3-thymidine (370 kBq/g b.w., i.p. and 185 kBq/ g b.w., s.c., resp.) and were killed 2, 6 and 24 hr and 3, 5 and 21 days post injection (p.i.).

Labelling index (L.I.) of germinative brain cells of both age groups (ED 16 and PD 5) amounted to 50 % at 6 hr p.i.The L.I. reached 95-98% and the grain count density (gr.c.d.) over the nuclei is similar (50-70 grains) at 24 hr p.i. The gr.c.d. decreased abruptly toward the 3th day p.i. (5-10 grains). At the 21st day p.i., the L.I. was still 45 %; the gr.c.d. was, however, very low ($\leq$ 3 grains). At 3 hr p.i., the total activity of H3-DNA (measured scintigraphically in PD 5 age group) reached its maximum between 6-24 hr p.i. and did not changed till the 21st day p.i.

It is concluded that (1) the single H3-thymidine injection was sufficient for labelling of nearly all germinative cells; this is because of longer availibility of radioactive precursor (-s) of DNA than earlier assumed (till $\leq$ 24 hr), (2) brain cells retaining medium amount of H3-DNA may have stopped division within 3 days p.i. This has to be taken into consideration in the studies of the non-neuronal cell formation where the total L.I. is often to be used instead of that based on heavily labelled nuclei.(1).

(1) D.Biesold, G.Brückner, V.Mareš (1976) Brain Res. 104:295-301.

A LONG-TERM FOLLOW-UP STUDY OF ^{3}H-DNA IN PURKINJE CELL NUCLEI OF THE MOUSE CEREBELLUM AFTER PRENATAL LABELLING WITH 3H-THYMIDINE

[1]V. Mareš, [2]E. Scherini, [3]B. Schultze-Maurer, [3]W. Maurer.
[1]Institute of Physiology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia; [2]Department of Animal Biology, University of Pavia, Pavia, Italy; [3]Institute of Medical Radiation Research, University of Würzburg, Würzburg, F.R.G.

Pregnant mice received ^{3}H-thymidine (5 µCi per g b.w., i.p.) and their offsprings were sacrificed on postnatal day 25 (PD 25), or at age 3, 6 or 9 months (M 3 to M 9). The ^{3}H-DNA in Purkinje cell nuclei (P.c.n.) was determined by counting silver grains in autoradiographed histological sections prepared from the cerebellar vermis. Dry mass concentration in P.c.n. was evaluated in parallel sections by interference microscopy and the area of P.c.n. by a graphic tablet.

The measured grain counts over the whole P.c.nucleus have not significantly changed till M 3; towards M 6 and M 9 a decrease by 14 % and 20 % respectively, was observed. The changes were more profound in the perinucleolar region of P.c.n. (-23 % and -29 % at M 6 and M 9, respectively. The decrease was apparent also after correcting grain counts for changes in the P.c.n. area, dry mass concentrations and natural decay of tritium during the experiment.

In agreement with a former study (1) most of the ^{3}H-DNA synthesized by Purkinje cell pre-neuroblasts at the time of their mitotic division is, therefore, retained in the nucleus. A significant loss is observed only after a longer survival (6 to 9 M). It might be due to a turnover or repair synthesis of DNA; the latter is higher in the region of the P.c. nucleolus.

(1) V.Mareš, B.Schultze-Maurer, W.Maurer (1974) J.Cell Biol. 63, 665, 1974.

AN INFLUENCE OF Db-cAMP AND CIS-DICHLORODIAMMINE PLATINUM (Cis-DDP) ON CHROMATIN STRUCTURE AND CELL CYCLE OF GLIOMA CELLS

[1]V. Mareš, [1]V. Lisá, [2]C. Pellicciari, [3]P. Giordano, [3]G. Mazzini, [1]J. Faltin, [2]E. Scherini, [4]J.Drobník.
[1]Institute of Physiology; [4]Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague, Czechoslovakia; [2]Department of Animal Biology, University of Pavia; [3]Center for Histochemical Study, C.N.R., Pavia, Italy.

C6-glioma cells were cultured for 3 days; then Db-cAMP (10^{-3}M) or cis-DDP (10^{-6}M to 10^{-7}M) were added for 24 h to 72 h. Some cultures were labeled with 3H thymidine. DNA was stained by Feulgen reaction or with propidium iodide.

Light-microscope-computerized microdensitometry revealed that the chromatin of cells treated with both drugs became less evenly distributed. Electron microscopy showed deep infoldings of the nuclear membrane in Db-cAMP treated cells. After cis-DDP accumulation of chromatin near the nuclear membrane was more apparent. Cells with more nucleoli were more frequent in treated cultures. Microdensitometry and cytofluorometry showed higher number of nuclei with G1-phase DNA content after Db-cAMP while after cis-DDP the nuclei with S- and G2-phase DNA content prevailed. In cis-DDP treated cultures higher number of unlabelled nuclei with S-phase DNA content appeared and the incorporation of 3H thymidine in cells passing through the S-phase decreased.

In conclusion, Db-cAMP caused a partial block of cell cycle at G1/S while cis-DDP at G2/S phases together with lower utilization of H3 thymidine for DNA synthesis. The chromatin changes might be due to higher phosphorylation of nuclear proteins; interaction with DNA molecule and higher frequency of cells in G2 phase might also contribute to cis-DDP effects.