

ORAL PRESENTATIONS

EFFECTS OF LIPOPOLYSACCHARIDE (LPS) ON CHLORPROMAZINE-INDUCED INHIBITION OF GLUCOCORTICOID RECEPTOR FUNCTION IN FIBROBLAST CELLS

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There is evidence that neuroleptic drugs and some cytokines influence expression and function of glucocorticoid receptor. In order to shed more light on the interaction of neuroleptics with immune and endocrine systems we investigated the effects of cytokine releasing agent, LPS, on chlorpromazine-induced inhibition of the glucocorticoid receptor-mediated gene transcription in fibroblast cells, stably transfected with an MMTV-CAT promoter (LMCAT cells). The effect of LPS on chlorproma-

zine-induced cytokine production and the reactivity of splenocytes was also studied. Both LPS (1–5 mg/ml) and chlorpromazine (3–10 μ M) dose-dependently decreased CAT activity. Co-incubation with chlorpromazine and LPS resulted in synergistic inhibitory effect on glucocorticoid receptor function.

These data indicate that activation of the immune system may sensitize the glucocorticoid receptors to inhibitory effects of neuroleptics.

SEROTONIN-INDUCED MODULATION OF SYNCHRONOUS ACTIVITY OF RAT FRONTAL CORTEX NEURONS *IN VITRO*

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The frontal cortex is innervated by serotonergic terminals from the raphe nuclei and it expresses di-

verse 5-HT receptors. A number of studies have been conducted to elucidate the effects of 5-HT on

cortical neurons and on the activity of cortical networks, however, their results are still conflicting. Both excitation and inhibition of cortical neurons have been reported, being possibly due to the activation of different 5-HT receptor subtypes. We investigated the effects of 5-HT and different 5-HT receptor subtype-selective agonists on spontaneous discharges which had developed in rat cortical slices perfused with a Mg^{2+} -free medium and the $GABA_A$ receptor antagonist picrotoxin. The frequency of synchronous discharges, recorded extracellularly in superficial layers of the frontal cortex, was dose-dependently enhanced by 5-HT (2.5–40 M). That excitatory effect was blocked by the 5-HT₂ receptor selective antagonist ketanserin. The

5-HT_{2A/2C} receptor-selective agonist DOI and the 5-HT₄ receptor agonist zacopride also increased the frequency of spontaneous discharges. In the presence of ketanserin, 5-HT decreased the discharge rate. A similar effect was observed when the 5-HT_{1A} receptor agonist 8-OH-DPAT or the 5-HT_{1B} receptor agonist CGS-12066B was applied. The 5-HT₃ receptor agonist m-CPBG was ineffective.

In conclusion, 5-HT produces multiple effects in the frontal cortex *via* activation of various 5-HT receptor subtypes. The excitatory action of 5-HT, which predominates, is mediated mainly by 5-HT₂ receptors. The inhibitory effects can be attributed to the activation of 5-HT_{1A} and 5-HT_{1B} receptors.

EFFECTS OF NEUROLEPTIC DRUGS ON GLUCOCORTICOID RECEPTOR-MEDIATED GENE TRANSCRIPTION

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This study examined the effect of various neuroleptic drugs on the glucocorticoid-mediated gene transcription in fibroblast cells, stably transfected with an MMTV-CAT promoter (LMCAT cells). Chronic treatment of cells with chlorpromazine (3–100 M) caused a concentration- and a time-dependent inhibition of the corticosterone-induced gene transcription. Also clozapine (3–100 M) evoked a significant decrease in glucocorticoid receptor-mediated gene transcription, but with lower potency. Among other drugs under study, only haloperidol, but at high concentration (30–100 M), inhibited glucocorticoid receptor function, while sulpiride, raclopride and remoxipride were without effect. The inhibitory effect of chlorproma-

zine (10 M) on the corticosterone-induced gene transcription was calcium-dependent, as shown by the ability of Ca^{2+} -ionophore (A-23187) to attenuate its action in statistically significant manner. Furthermore, the effect of chlorpromazine was significantly diminished by phorbol ester (TPA), PKC activator.

It is concluded that some neuroleptics are able to inhibit glucocorticosteroid receptors action. Moreover, our data suggest that mechanism of inhibitory effect of chlorpromazine on the corticosterone-induced gene transcription involves Ca^{2+} dependent processes, especially inhibition of PKC activity.

EFFECT OF BACLOFEN AND AIDA ON BEHAVIOR OF RATS AFTER HYPOXIA

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The central nervous system and its high level functions, including learning and memory, are most vulnerable to hypoxia. In the present study, we investigated the effect of baclofen and AIDA on behavior in rats after hypoxia, as a model of experimentally induced amnesia. Under physiological circumstances, baclofen stimulated locomotion, did not influence consolidation and retrieval of conditioned avoidance and had anxiogenic effect. AIDA produced inhibition of locomotor activity, enhanced consolidation process, had no effect on retrieval or in elevated "plus" maze. Hypoxia reduced locomotor activity, impaired consolidation and retrieval processes and produced anxiogenic effect in

the elevated "plus" maze. Hypoxic circumstances evoked the following changes in comparison with physiological conditions: baclofen reduced locomotion, improved consolidation, produced anxiolytic activity, while AIDA enhanced reduction of locomotor activity, produced anxiolytic effect in the elevated "plus" maze.

In summary, baclofen and AIDA did not change reduction of locomotor activity obtained after hypoxia, did not influence hypoxia-induced impairment of retrieval process but prevented consolidation deficit induced by hypoxia and reduced anxiogenic effect caused by amnesia.

IMMUNOHISTOCHEMICAL STUDY OF DOPAMINE D1 RECEPTORS EXPRESSED IN MAGNOCELLULAR NEURONS OF RAT PARAVENTRICULAR NUCLEUS OF HYPOTHALAMUS

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The paraventricular nucleus of hypothalamus (PVN) plays an important role as a neuronal interface between various brain structures and endocrine system. It receives substantial and homogeneous dopaminergic innervation. In the past few years several data showed the presence of D1 receptors as well as their mRNA in the PVN. In order to define cellular localization of dopamine D1 receptor protein in the abovementioned brain structure and to establish anatomical and hormonal characterization of PVN compartments containing D1 receptors, an immunohistochemical technique was used with highly specific monoclonal antio-

body recognizing: C-terminal fragment of D1 receptor and hypothalamic neuropeptides, i.e. vasopressin and oxytocin. Dopamine D1 receptors were found in numerous neurons of the PVN, especially in its magnocellular part. Immunolabelling was mainly restricted to neuronal perikarya, however, occasionally faintly stained dendritic processes were also observed. In general, magnocellular neurons of PVN are known to contain vasopressin and oxytocin and they send projections to the neural part of pituitary gland (neurohypophysis). In the present study, analysis of a series of consecutive PVN sections immunostained for D1 receptors, va-

sopressin or oxytocin revealed that these receptors and neuropeptides have very similar pattern of anatomical distribution.

The above-described results suggest that D1 receptors are probably expressed in vasopressin- and oxytocin-positive neurons, however, this conclusion should be subjected to further confirmation using

double-labeling immunohistochemical or immunofluorescence methods. The presence of D1 receptors in the paraventricular nucleus of hypothalamus indicates (at the anatomical level) that these receptors are possibly engaged in the regulation of endocrine system activity, e.g. by controlling the synthesis or/and release of vasopressin and oxytocin.

IMPACT OF CHRONIC CORTICOSTERONE TREATMENT ON DOPAMINERGIC NEUROTRANSMISSION

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In recent years, there is a growing interest in the central effects of glucocorticoids strengthened by clinical and experimental observations that these hormones may increase sensitivity and vulnerability to the effects of addictive psychostimulants. A special interest has been paid to the impact of glucocorticoids on the dopaminergic systems, since the dopaminergic neurotransmission has been regarded as the most important factor in the phenomenon of addiction and drug-induced psychoses. Several observations suggest that glucocorticoids might contribute to the increases in dopaminergic activity. Therefore, in order to find further arguments that glucocorticoids are capable to influence the dopaminergic neurotransmission, in our present study we investigated the effect of prolonged treatment with corticosterone (10 mg/kg *sc* twice daily, for 7 days) on the biosynthesis of tyrosine hydroxylase, the rate limiting enzyme in dopamine synthesis. The amount of enzyme protein was measured using Western blot method, and the level of mRNA was evaluated by the method of *in situ* hybridization. In addition, the effect of corticosterone on do-

pamine metabolism was evaluated. We found that corticosterone increased the level of mRNA encoding tyrosine hydroxylase in the substantia nigra and ventral tegmental area of the rat brain. We also observed that the level of the enzyme protein was slightly increased in the nucleus accumbens. In the next experiment, we found that the levels of dopamine, DOPAC and HVA in the ventral tegmental area were significantly decreased by corticosterone, whereas there were no changes in the striatum and nucleus accumbens. However, chronic treatment with corticosterone decreased significantly the level of dopamine and its metabolites in the prefrontal cortex.

Our present results indicate that corticosterone may increase dopaminergic neurotransmission by increasing biosynthesis of tyrosine hydroxylase. At the same time, some aspects of dopaminergic activation might be attenuated by decreasing dopamine levels. These two opposite effects may explain the discrepancy existing in the literature indicating that some effects of dopamine agonists are enhanced while other are attenuated by glucocorticoids.

EFFECTS OF SINGLE AND REPEATED ADMINISTRATION OF TIANEPTINE AND FLUOXETINE ON THE CENTRAL DOPAMINERGIC SYSTEM RECEPTORS: AUTORADIOGRAPHY AND *IN SITU* HYBRIDIZATION STUDY

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The effects of two antidepressant drugs (ADs) with opposite pharmacological profile, tianeptine (TIA 5 and 10 mg/kg *po*), a serotonin reuptake enhancer, and fluoxetine (FLU 10 mg/kg *po*), a serotonin reuptake inhibitor, on dopaminergic receptors were compared after single and repeated administration (twice daily for 14 days) in the rat brain. We have previously reported that TIA and FLU, administered repeatedly, potentiate behavioral effects evoked by dopamine receptor stimulants. The aim of the present study was to establish whether TIA and FLU administered repeatedly induce changes at the level of dopamine receptors similar to those produced by tricyclic ADs.

This study used *in situ* hybridization to examine the effect of TIA and FLU on the levels of mRNA encoding dopamine D₁ and D₂ receptors, and receptor autoradiography, using [³H]raclopride and [³H]spiperone (D₂/D₃ antagonists), [³H]quinpirole (D₂/D₃ agonist), [³H]7-OH-DPAT (D₃ agonist) and [³H]SCH23390 (D₁ antagonist). Dopamine receptors were studied in the nucleus accumbens (shell and core), caudate putamen and islands of Calleja.

The obtained results show that TIA and FLU administered repeatedly decreased the level of D₁ mRNA in the nucleus accumbens shell but not in the other brain regions. The same effect was observed for D₂ mRNA expression in the nucleus accumbens shell and core (except for TIA 10 mg/kg). FLU also decreased the level of D₂ mRNA in the caudate putamen. Autoradiographic analysis showed that TIA and FLU after repeated administration increased [³H]quinpirole binding in the nucleus accumbens core and caudate putamen. In the islands of Calleja, TIA and FLU decreased [³H]quinpirole and [³H]7-OH-DPAT binding. Binding of [³H]SCH23390 was also decreased in the nucleus accumbens following FLU (but not TIA) administration. [³H]spiperone binding was not changed. In contrast, [³H]raclopride binding was increased following TIA 5 and FLU 10 in the nucleus accumbens core and caudate putamen.

Relevance of the obtained results to the effects of other ADs will be discussed.

ETHANOL VOLUNTARY CONSUMPTION IN RATS BRED SELECTIVELY FOR DIFFERING PREFERENCE FOR ETHANOL: INFLUENCE OF THE INITIATION PROCEDURE

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The selectively bred ethanol-preferring and non-preferring lines of rats have been frequently

used to study the mechanisms underlying ethanol drinking and dependence. In our laboratory, new

lines of Wistar rats have been selectively outbred for more than 7 years, and 19th–20th generations of high ethanol preferring rats (WHP-Warsaw High Preferring) are currently available [1, 2]. After the first selection procedure, the highest scoring females and males were used in order to initiate upward selection, while the lowest scoring pairs were used to initiate downward selection. In order to estimate the ethanol intake and preference, the rats were individually housed in wire cages containing two graduated drinking tubes. During the entire experiment the rats had free access to standard laboratory chow. The animals were presented with 10% ethanol (v/v) solution and water (two-bottle choice test) for 4 weeks. The results (19th–20th generations) have shown that mean ethanol intake in WHP rats was higher than 5.0 g/kg/24 h while male WLP (Warsaw Low Preferring) rats consumed generally less than 2.0 g/kg/24 h. However, when the WLP rats were given access to gradually increasing

concentration of ethanol solutions, the ethanol intake increased. This procedure consisted in gradually increasing solution concentration (2, 3, 4, 5, 6, 7, 8, 9 and 10%). Each session lasted one week. Generally, similar result was obtained when outbred Wistar males were presented with different initiation procedure described above. Taken together, it appears that ethanol can function as a reinforcer not only for WHP rats but also for WLP rats depending on initiation procedure.

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EFFECT OF SELECTIVE ADENOSINE A_{2A} RECEPTOR ANTAGONISTS ON DOPAMINE RELEASE IN THE RAT STRIATUM

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Due to the key role played by adenosine A_{2A} receptors in the regulation of striatal dopaminergic neurotransmission, drugs acting on these receptors are likely to be useful in the treatment of neurological disorders related to dopaminergic dysfunction, in particular Parkinson's disease. Adenosine A_{2A} receptor antagonists have recently been proposed as potential new agents for treating this disease.

In the present study, we have examined the effect of new selective adenosine A_{2A} receptor antagonists with xanthine and non-xanthine chemical structure: 8-(3-chlorostyryl)caffeine (CSC) and (4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-yl-amino]ethyl)phenol) (ZM 241385) on dopamine (DA) release in rat striatum using *in vivo* microdialysis. Extracellular level of DA and its me-

tabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) was assayed by HPLC-ED. ZM 241385 applied locally into the striatum (100 mM) decreased extracellular concentration of DA and its metabolites DOPAC and HVA. However, systemic administration of ZM 241385 (1 mg/kg *ip*) did not affect striatal level of DA and its metabolites. CSC, the compound with a poor water solubility, given only peripherally (1 and 5 mg/kg *ip*) decreased extracellular concentration of DA and lowered DOPAC level at a higher dose.

These findings suggest that adenosine A_{2A} receptor antagonists can modulate activity of DA nigrostriatal pathway interacting with adenosine A_{2A} receptors expressed in striatopallidal neurons.

STUDIES ON ANXIETY DURING WITHDRAWAL FROM CHRONIC ADMINISTRATION OF DRUGS OF ABUSE IN RATS

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An increase in the level of anxiety is one of the clinically significant abstinence symptoms observed in people during withdrawal from chronic drug abuse. However, in contrary to physical symptoms, changes in anxiety associated with withdrawal following chronic use of psychoactive substances have not been well examined in animals. Therefore, the present study was designed to characterize the behavior of male rats withdrawn from chronic administration of various drugs of abuse in an elevated plus-maze test. This test is frequently used to evaluate changes in anxiety in animals, and accordingly, we found that an acute dose of diazepam (2.5 mg/kg) and picrotoxin (2 mg/kg) substantially increased and decreased the open arm entries, respectively, without changing locomotor activity measured by the total number of entries. Animals were administered twice daily with morphine (10 mg/kg), nicotine (2.5 mg/kg), diazepam (10 mg/kg), cocaine (10 mg/kg) or amphetamine (1.5 mg/kg) for two weeks. This administration procedure has been

previously shown to induce physical symptoms of withdrawal precipitated by naloxone (2 mg/kg), mecamylamine (2 mg/kg) or flumazenil (15 mg/kg); 24 and 72 h after the last injection, the animals were put in the middle of the maze and the number of entries into the open and closed arms was measured in a 5-min test. It was found that at both time points, the open arm entries were substantially decreased in animals having received chronic diazepam, cocaine and amphetamine treatment. Animals injected with morphine and nicotine also showed lower exploration of the open arms but this effect was smaller and did not reach statistical significance.

These results indicate that animals withdrawn from chronic administration of psychoactive substances show increase in their level of anxiety, and that this effect appears to be independent of the pharmacological profile of the drugs used to induce dependence.

INFLUENCE OF HEAVY METALS ON THE FLASH VISUAL EVOKED POTENTIALS (FVEP) IN RATS AFTER PRENATAL INTOXICATION

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Some metals are known as neurotoxic, because of their deleterious effects on the nervous system. The most noticeable neurotoxic metals, such as manganese (Mn), mercury (Hg), lead (Pb), cadmium (Cd), induce the neurological alterations and disturbances of the neurotransmitter function in the nervous system. The purpose of this study was to

find out if any and how deep alterations in visual tract are due to prenatal intoxication by heavy metals such as Cd, Pb, Hg and Mn. The measure of it were the changes in flash visual evoked potentials after prenatal intoxication.

Methods. The experiments were carried out on 55 white rats stereotaxically implanted with poly-

ethylene cannulas into the lateral brain ventricle (*icv*), with active electrode under and reference one on the skull; 7 days later the FVEP were recorded by the 1000 LKC electrophysiologically interfaced personal computer system (USA), with ganzfield stimulation of both mydriatic eyes, under chloral hydrate anesthesia. The animals were intoxicated in the Department of Pharmacology in Zabrze and divided into 5 groups: control group (14 rats) and groups treated with Cd (12), Pb (6), Hg (14) and Mn (9).

Results. LATENCY of FVEP. The latencies of the peaks N_1 and P_2 were prolonged in the Mn group up to 113–118% ($p < 0.05$). Slight prolongation of N_1 latency by about 1% was statistically significant in Cd and Hg groups ($p < 0.05$), but in Pb group prolongation by 4% was not statistically sig-

nificant. The differences in P_2 latencies were not statistically significant in these groups compared to the control. AMPLITUDE of FVEP. The amplitude of N_1 wave decreased in Cd group by about 63% and in Mn group by 32% compared to the control ($p < 0.05$). In Hg-intoxicated group, the N_1 amplitude decreased to 56% ($p < 0.01$). The amplitude of P_2 statistically significantly decreased in all intoxicated groups (Hg to 56%, Cd to 55%, Mn to 49%), except for the Pb group in which even a 21% decrease was not significant.

Conclusion. The changes of FVEP after prenatal intoxication were observed in all groups. The heavy metals (Mn, Hg, Cd, Pb) caused the delay of latencies and diminution of the amplitudes of flash visual evoked potentials, so they disturb visual transmission (ability to vision).

L-AP4, A POTENT AGONIST OF GROUP III METABOTROPIC GLUTAMATE RECEPTORS, DECREASES ANGIOTENSIN II-INDUCED BEHAVIOR

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The role of the class III metabotropic glutamate receptors (mGluRs) in the behavioral activity of angiotensin II (AngII) was investigated in the present study. The experiments were performed on adult male Wistar rats. Stimulation of the group III mGluRs was evoked by *icv* injection of an agonist, L-2-amino-4-phosphonic acid (L-AP4) (80 mM 5 L^{-1}). Fifteen minutes later, the animals were given *icv* injection of the solution containing 1 nmol of AngII. Memory motivated affectively was evaluated as passive avoidance and active avoidance (CARs) responses. Moreover, the speculative influence of the treatment on motor activity was tested in open field. We observed that both com-

pounds did not have significant influence on motor activity of rats in open field test. L-AP4 given alone, had no influence on acquisition, consolidation and recall of a passive avoidance responses. Examination of the influence of L-AP4 on the acquisition and extinction of CAR proved that this compound decreased acquisition of CARs, while it did not alter extinction of these responses. AngII, as repeatedly shown before, greatly increased passive avoidance latency, rate of acquisition of CARs and decreased extinction. The pretreatment of rats with L-AP4 prevented all above behavioral effects of the AngII administration.

EFFECT OF NEW PUTATIVE 5-HT_{1A} SEROTONIN RECEPTOR AGONISTS ON SEROTONIN AND DOPAMINE RELEASE IN RAT PREFRONTAL CORTEX

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Some piperazine derivatives displaying *in vitro* high affinity for serotonin 5-HT_{1A} receptor have a potential as anxiolytic and antidepressant drugs.

In the present study, we have examined an effect of 1-[4-(4-quinolin-2-yl-piperazin-1-yl)butyl]-piperid-2-one (MC1) and 1-[4-(2-methyl-4-quinolin-2-yl-piperazin-1-yl)butyl]-8-azaspiro[4.5]decane-7,9-dione (MM5) on spontaneous release of serotonin (5-HT) and dopamine (DA) in the rat prefrontal cortex using *in vivo* microdialysis. The extracellular level of 5-HT, DA and their metabolites: 5-hydroxyindoleacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid

(HVA) were measured by HPLC with electrochemical detection. It was found that MM5 (30 mg/kg *ip*) decreased 5-HT and 5-HIAA level, but did not exert an effect on DA release. On the other hand, MC1 (30 mg/kg *ip*) did not influence 5-HT release, but increased extracellular level of DA, DOPAC and HVA.

The presented results indicate that MM5 (similarly to 8-OH-DPAT) exhibits 5-HT_{1A} agonist action on 5-HT release. On the other hand, MC1 represents a drug with dopaminergic profile devoid of 5-HT agonist activity.

EFFECT OF NITRIC OXIDE SYNTHASE INHIBITOR, L-N^G-NITRO-L-ARGININE, ON FORCED SWIM TEST AND NEUROTRANSMITTERS METABOLISM IN MICE FRONTAL CORTEX AND HYPOTHALAMUS

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Our previous experiments have demonstrated that nitric oxide synthase (NOS) inhibitors have antidepressant-like activities in mice forced swim test, and after chronic treatment produce α -adrenergic receptor down-regulation. The aim of present study was to investigate the effect of NOS inhibitor on dopamine (DA) and serotonin (5-HT) metabolism. Mice were treated acutely with imipramine (15 mg/kg *ip*), NOS inhibitor L-N^G-nitro-L-arginine (L-NA, 0.5, 1, 3, 5, 10, 30 mg/kg *ip*) and electroconvulsive shock (ECS). Experiments were carried out 1 h after drug injection. Metabolism of DA

and 5-HT was investigated in the frontal cortex and hypothalamus using high performance liquid chromatography (HPLC) with electrochemical detection, forced swim test (FST) was performed using protocol described previously by Porsolt et al. (1977). In the present study, we have observed the biphasic, U-shaped effect of L-NA on the forced swim test with effective doses of 1, 3, 10 mg/kg, while intermediate doses of 0.5, 5, 30 mg/kg failed to produce the same effect. The effect of NOS inhibitor L-NA was reversed by NOS substrate L-arginine what indicates that NO is involved in the

mechanism active in FST. L-NA at doses possessing activity in FST (1 and 10 mg/kg) decreased the level of DA (by 25%, $p < 0.05$, and 28% $p < 0.05$, respectively) and its metabolite DOPAC (25%, $p < 0.05$). Additionally, L-NA (1 mg/kg) given acutely increased the level of 5-HT in the frontal cortex (by 34%, $p < 0.05$), while L-NA treatment (10, 30 mg/kg) elevated the level of 5-HT metabolite 5-HIAA in the hypothalamus (by approximately 30%, $p < 0.05$). Imipramine and ECS given

acutely failed to change the DA metabolism, however imipramine (15 mg/kg) given acutely produced massive increase in the level of 5-HT in the frontal cortex as well as in the hypothalamus (by 40%, $p < 0.01$). Thus, it appears that under basal conditions endogenous NO may play a role in positive control of the level of DA and negative control of the level of 5-HT, and inhibition of this interaction by NOS inhibitor L-NA may result in antidepressant-like effect in forced swim test.

INFLUENCE OF SULPHUR-CONTAINING AMINO ACIDS ON KYNURENIC ACID PRODUCTION *IN VITRO*

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Kynurenic acid (KYNA) is an endogenous brain constituent that inhibits the activity of all three ionotropic excitatory amino acid (EAA) receptors. Cerebral synthesis of KYNA from its bioprecursor L-kynurenine is catalyzed by aminotransferases (KATs) localized preferentially within astrocytes. The possible role of altered KYNA-mediated modulation of EAA receptors in the human neuropathology has been postulated.

In the present study, the influence of sulphur-containing amino acids on KYNA synthesis in the rat brain cortex and their influence on KAT I and

KAT II activities was determined. All investigated sulphur-containing amino acids, e.g. cysteine sulphonic acid, cysteine-S-sulphinic acid, homocysteine sulphonic acid, cysteic acid and homocysteic acid reduced KYNA production in the rat brain slices and lowered preferentially the activity of KAT II in a dose-dependent manner.

The obtained data suggest that endogenous sulphur-containing amino acids can modulate KYNA production in the brain.

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DEXTROMETHORPHAN, MEMANTINE AND MRZ 2/579, LOW AFFINITY NMDA RECEPTOR ANTAGONISTS, POTENTIATE MORPHINE ANTINOCICEPTION RECORDED FROM THE TAIL BUT NOT FROM THE PAW

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We investigated the effect of low affinity, non-competitive NMDA receptor antagonists on morphine-

ne-induced antinociception in rats when the same nociceptive stimulus was used for the tail and the paw.

The same nociceptive thermal (radiant heat) stimulus produced similar baseline response from the tail and from the paw in male Wistar rats. However, to achieve similar antinociceptive effect in the tail and the paw rats were treated (*sc*) with 2.5 mg/kg of morphine in the tail studies and 6 mg/kg of morphine in the paw studies. NMDA receptor antagonists were administered (*ip*) 30 min before morphine: dextromethorphan [DXM] at doses of 2.5, 5, 15 and 30 mg/kg, memantine [MEM] at doses of 2.5, 5, 10 and 15 mg/kg and MRZ 2/579 [MRZ] at doses of 1.25, 2.5, 5 and 10 mg/kg. Morphine antinociception was measured for 210 min, in 30 min intervals. In the tail studies, DXM, MEM and MRZ given alone (at doses of 30, 10 and 10 mg/kg, respectively) had no antinociceptive effect but they all significantly and dose-dependently po-

tentiated morphine-induced antinociception. In the paw studies DXM, MEM and MRZ given alone (at doses of 30, 10 and 10 mg/kg, respectively) also had no antinociceptive effect and when given before morphine did not affect its effect.

The data suggest that the sensitivity to noxious stimulation is similar for the tail and the paw when the same intensity of nociceptive stimulus is used. However, there is distinct morphine antinociceptive effect and the distinct influence of NMDA receptor antagonists on the tail and the paw. This indicates differences in neuronal pathways mediating the tail and the paw antinociception. Additionally, it may explain contrasting results in some previous studies concerning the influence of NMDA receptor antagonists on morphine antinociception in tail-flick and hot plate paradigms.

ANTIDEPRESSANT-LIKE EFFECTS OF ZINC IN RODENT SCREENING TESTS AND IN OLFACTORY BULBECTOMY MODEL OF DEPRESSION

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Zinc is a very potent inhibitor of the NMDA receptor complex and plays an important role in a wide range of biochemical processes. Recent studies have indicated, that zinc may be involved in the mechanism of antidepressant therapy. Lower zinc blood concentration was found in depressed patients, which was normalized after successful antidepressant therapy. Moreover, chronic electroconvulsive shock (ECS) treatment increased zinc concentration in the hippocampus.

In the present experiments, we studied the effects of zinc in mouse and rat forced swimming test, in passive avoidance test in the olfactory bulbectomized rat model of depression and in biochemical studies. Zinc (zinc sulphate) at a dose of 30 mg/kg (12 mg of zinc) similarly to imipramine (30 mg/kg) reduced the immobility time in forced

swimming test in mice. Both doses of zinc reduced the locomotor activity in mice. Zinc (zinc hydroaspartate) at a dose of 65 mg/kg (12 mg of zinc) was also active in a forced swimming test in rats. Acute treatment with zinc (zinc hydroaspartate) at a dose of 65 mg/kg improved passive avoidance performance of bulbectomized rats. Our biochemical studies indicated that chronic antidepressant treatment increase the potency of zinc to inhibit the NMDA receptor activity (³H]MK-801 binding to NMDA ionophore) in mouse cerebral cortex.

The obtained results indicate that zinc induces antidepressant-like effects in rodents and support the notion that inhibition of the NMDA receptor function is involved in the mechanism of antidepressant action.

EFFECTS OF LIGANDS OF 5-HT_{1A} AND 5-HT₂ RECEPTORS ON CYTOKINE PRODUCTION

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Serotonin (5-HT) is a neurotransmitter and an immune modulator. Antidepressants with a serotonergic mode of action have negative immunoregulatory effects *in vitro*. We have hypothesized that part of these effects may be explained by the serotonergic actions of antidepressants on immunocytes. This study was carried out to examine the effects of serotonergic agonists and antagonists on the production of interferon- γ (IFN- γ), a pro-inflammatory cytokine and interleukin 10 (IL-10), a negative immunoregulatory cytokine by whole blood stimulated with mitogens. Blood was obtained from younger and older normal volunteers, and patients with treatment-resistant major depression, treated with fluoxetine for at least 6 weeks. We found significantly higher production of IFN- γ in depressed patients than in age-matched (older) normal volunteers. The observed increase in IFN- γ production is in agreement with previous findings showing that inflammatory response is more pro-

nounced in patients with treatment-resistant depression despite antidepressive treatment. Co-incubation of blood with serotonergic substances showed that: 1) fleroxan (a 5-HT_{1A} agonist) had no significant effects on the production of above cytokines; 2) 8-OH-DPAT (a 5-HT_{1A} agonist) and WAY-100635 (a 5-HT_{1A} antagonist) inhibited IFN- γ production and suppressed IFN- γ /IL-10 ratio (for younger volunteers, other groups were not tested); 3) mCPP (a 5-HT_{2A/2C} agonist) and ritanserin (a 5-HT_{2A/2C} antagonist) decreased IFN- γ /IL-10 ratio, and ritanserin inhibited IFN- γ production.

Summing up, these data indicate that 5-HT_{1A} and 5-HT_{2A/2C} receptors are not involved in antidepressant-induced IL-10 production. On the other hand, it appears that serotonergic mechanism plays a role in the inhibition of IFN- γ production and/or decrease in IFN- γ /IL-10 ratio following antidepressants treatment.

AMPA RECEPTOR-MEDIATED REGULATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) IN PRIMARY NEURON CULTURES

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Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors that promote the growth and development of immature neurons as well as enhance the survival and function of adult neurons. Recent studies have indicated that mRNA encoding BDNF, the

most abundant neurotrophin in the brain, is induced following chronic, but not acute treatment of rats with many clinically effective antidepressants (Nibuja et al., 1995). The important role for BDNF in the pathogenesis and treatment of major depression has been also proposed by Duman et al. (1997).

Hayashi, et al. [Nature 1999, 397, 72–76] demonstrated that Lyn, (a member of the Src-family of non-receptor protein tyrosine kinases) is physically and functionally associated with AMPA receptors in mouse cerebellar granule neurons. Further, in these cultured neurons, BDNF gene expression is regulated by Lyn-mediated activation of MAPK in response to AMPA receptor signalling.

In the present study, we determined if activation of AMPA receptors, using the novel AMPA receptor modulator, LY392098, is able to induce BDNF expression in primary neuron cultures from both the cortex and cerebellum. Neurons were prepared from the cerebella of 8-day-old rat pups and cerebral cortices from E17 rats, and were cultured for 8–12 days. BDNF mRNA levels were determined by Northern blot analysis. BDNF protein was determined using the BDNF Emax ImmunoAssay System. Concentration and time course studies performed after the addition of AMPA and/or LY392098 to the culture medium revealed that BDNF mRNA levels were dramatically elevated. The most effective concentrations of each compound following a 6 h incubation increased BDNF

mRNA in cerebellar granule cells (~1–1.5 fold) and in cortical neurons (AMPA ~24 fold; LY392098 ~7) vs. untreated control. Moreover, co-addition of subeffective concentrations of AMPA (1 μ M) and LY392098 (1 μ M) to cortical neuronal cultures resulted in a remarkable (~45 fold) increase in BDNF mRNA levels. However, in granule cell neurons co-addition of AMPA (1 μ M) and LY392098 (5 μ M) produced increase in BDNF mRNA to a maximum of ~2.5 fold. Increases in BDNF protein were paralleled by increases in BDNF mRNA in cerebellar granule cells, but in cortical neurons not as robust. These effects were blocked by the AMPA receptor antagonist, NBQX, but not by the NMDA antagonist, MK-801 indicating that the expression of BDNF in these neurons is regulated by activation of AMPA receptors. In cortical neuron cultures, activation of both L-type Ca^{+2} channels and mitogen-activated protein (MAP) kinases contribute to AMPA receptor-mediated increases in BDNF mRNA. The ability of LY392098 to increase the expression of BDNF in primary neuron culture indicates this and related biarylpropylsulfonamides may be useful in the treatment of neuropsychiatric disorders.

EFFECT OF SOME NEUROSTEROIDS ON COCAINE-INDUCED KINDLING IN MICE

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Neurosteroids are potent modulators of nervous system activity and some of them have anticonvulsant properties.

The present study investigated the effects of neurosteroids representing various pharmacological groups on cocaine-induced seizures. We found that 5 α -pregnan-3 α -ol-20-one (5 mg/kg, *ip*) and dehydroepiandrosterone sulfate (20 mg/kg, *ip*) inhibited development of cocaine-induced kindling (45 mg/kg, *ip*, once a day for 12 days). A positive

modulator of GABA_A receptor, 5 α -pregnan-3 α -ol-20-one (5 mg/kg, *ip*), tended to inhibit cocaine-induced kindling but this effect was not significant. None of these steroids affected seizures induced by a single high dose of cocaine (75 mg/kg, *ip*).

These data indicate that some neurosteroids can inhibit sensitization to toxic effects of chronic cocaine and that these effects are not correlated with GABA_A agonistic activity.

INFLUENCE OF METHOXAMINE INJECTED INTO THE LATERAL GENICULATE NUCLEUS (LGN) ON VISUAL TRANSMISSION (FLASH VISUAL EVOKED POTENTIALS (VEPs) IN RATS

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Purpose. It has been found in our previous study that norepinephrine injected into the lateral geniculate body intensifies visual transmission in the rat brain. The aim of the present study is to determine the effect of methoxamine (specific α -receptor agonist) injected into LGN on VEP.

Method. The experiments were performed on female adult Wistar rats weighing 220–325 g. Under chloral hydrate anesthesia (0.3 ml/100 g *ip*) the rats were stereotaxically implanted with electrodes: active electrode under the skull on dura mater in occipital region of the brain and reference one on the skull in the interorbital region. Moreover, guide canula was implanted 2 mm above the planned site of injection into GL. After next 5–7 days, the flash visual evoked potentials was recorded by the 1000 LKC electrophysiologically interfaced personal com-

puter system (USA), with Ganzfeld stimulation of both mydriatic eyes (1% mydriacyl and 1% atropine), under the chloral hydrate anesthesia. Every 5 min of the experiment we analyzed VEP curve before and after injection of 1 μ l of 0.9% NaCl and methoxamine at doses of 1 and 5 nmol into LGN. We have calculated amplitudes and latencies of negative peak N1 and positive P2 following it.

Results. After methoxamine treatment, the amplitudes of N1 were increased up to 135%, and amplitudes of P2 were increased up to 120%. The latencies of N1 and P2 were shortened about 4% and 5%, respectively.

Conclusion. The results of this study show that methoxamine intensifies visual transmission in the rat brain by influencing α -adrenergic receptors in the lateral geniculate body.

EFFECT OF REPEATED TREATMENT WITH REBOXETINE ON THE CENTRAL DOPAMINERGIC SYSTEM IN THE RAT

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Reboxetine (REB) is a new potent antidepressant drug (AD), selective noradrenaline reuptake inhibitor with no affinity for serotonin or dopamine transporters. In contrast to typical tricyclic ADs, REB shows no affinity for various central neurotransmitter receptors nor does it inhibit the rat brain monoamineoxidase A or B. The clinical efficacy of

REB is comparable to that of typical tricyclics (like desipramine or amitriptyline).

Our earlier studies showed that ADs administered repeatedly, but not at a single dose, potentiated behavioral effects (locomotor hyperactivity) evoked by dopamine stimulants, such as D-amphetamine, quinpirole or 7-OH-DPAT. Those find-

ings indicate that ADs given repeatedly activate the dopaminergic system. Further support to this concept comes from the biochemical studies, which show that repeated administration of ADs increases the binding of ligands specific for dopamine D₂ and D₃ receptors in various regions of the rat brain.

The present study was aimed at determining whether REB evokes, when given repeatedly, the changes similar to those induced by tricyclic drugs. To this end, we administered REB (10 or 30 mg/kg *po* acutely or repeatedly, i.e. twice daily for 14 days) and studied the behavioral response of rats to the agonists of dopamine D₂ and D₃ receptors. The obtained results showed that REB administered repeatedly (30 but not 10 mg/kg) increased the rat locomotor hyperactivity induced by D-amphetamine or 7-OH-DPAT (but not by quinpirole), measured at 24 h after the last dose.

In biochemical experiments we used the autoradiography of dopamine receptors with the radioligands of various specificity: [³H]raclopride (D₂/D₃ receptor antagonist), [³H]quinpirole (D₂/D₃ receptor agonist) and [³H]7-OH-DPAT (D₃ receptor agonist), as well as *in situ* hybridization to measure the level of mRNA coding for dopamine D₁ and D₂ receptors in different regions of the rat brain. Biochemical data indicate that neither acute nor repeated treatment with REB (10 or 30 mg/kg) induced any statistically significant alterations in the binding or expression of dopamine receptors in the rat brain.

The behavioral and biochemical effects of REB will be discussed in comparison to the effects induced by typical tricyclic ADs.

EFFECT OF IFENPRODIL ON THE DISTRIBUTION OF PERIPHERAL BLOOD LYMPHOCYTE SUBSETS AND TRANSFERRIN MICROHETEROGENEITY IN RATS CHRONICALLY TREATED WITH ETHANOL

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It is postulated that ifenprodil (IF) has some antialcoholic properties [4]. The purpose of this study was to assess the effect of multiple (21×) IF (1.0 mg/kg, *ip*) treatments on the composition of the peripheral blood lymphocyte subsets, transferrin levels (TF) and its microheterogeneity (MHT) using Warsaw High Preferring (WHP), Warsaw Low Preferring (WLP) and control, ethanol-naive Wistar (CR) rats. The number of lymphocyte subsets (CD3, CD4, CD8, CD161, CD45RA, CD25, CD71) was measured by flow cytometry method [3]; TF and MHT (3 variants) were assayed using the affinity immunoelectrophoretic methods [1, 2]. IF increased percentage of total lymphocytes in

WHP and WLP animals leading to normal values obtained in CR rats. A normalization of percentage of CD71⁺ (transferring receptor) cells after IF administration in WHP animals was also observed, while the rest of subsets were unaffected by IF. IF treatment led to the similar changes of MHT both in WHP and CR rats. No such alterations in WLP animals were found. On the contrary, after IF treatment the lowering of TF in CR rats was observed only.

Concluding, the possible role of IF in the modulation of ethanol-induced response expressed by the lymphocyte subset distribution and MHT should be considered.

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EFFECT OF AMPHETAMINE AND QUINPIROLE
ON THE RELEASE OF DOPAMINE AND ITS METABOLITES
IN THE BRAIN OF NEONATAL QUINPIROLE-PRIMED RATS.
AN *IN VIVO* MICRODIALYSIS STUDY

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Sensitization of central dopamine (DA) receptors is consistently achieved by repeated injections of DA agonists or dopaminomimetics such as amphetamine (AMPH) or cocaine. Increased reactivity of DA receptors is manifested behaviorally as enhanced agonist-induced stereotypy and locomotor activity. Ontogenic quinpirole priming of rats, from 1st to 11th days after birth (50 g/kg/d), is manifested as the increased quinpirole-induced yawning and locomotor effects throughout their whole adult life [1, 3]. Despite these obvious signs of DA receptor supersensitivity, there is no alteration of binding parameters (B_{max} , K_d) in the brain [2]. However, by means of *in vivo* microdialysis, we have found that AMPH (1.0 mg/kg *ip*) acutely induces a 5-fold increase in DA release in the neostriatum of rats that had been quinpirole-primed ten days after birth. This effect was accompanied by a reduction in DOPAC and HVA levels in the microdialysate. On the other hand, acute injection of quinpirole (0.1 mg/kg *ip*) reduced DA, DOPAC and HVA release in the brain microdialysate and the effect was comparable in the control and quinpirole-primed rats. The exaggerated effect of AMPH in quinpirole-primed rats indicates that there is a cascade of neuronal alterations accompa-

nying D_2 receptors priming, and this reorganization is reflective of a neurotoxicity which is not necessarily related to necrosis or apoptosis. Conceivably, a permanent alteration of neural responsiveness, resulting in enhanced DA overflow (i.e. in the synaptic space) could have additional deleterious consequences, relating to initiation of Fenton chemistry and formation of reactive oxygen species such as hydroxyl radical (HO^{\bullet}) and neurotoxic catecholquinones.

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POSTMORTEM DETERMINATION OF CATECHOLAMINES IN THE RAT BRAINS, KEPT IN -70°C , $+4^{\circ}\text{C}$ AND $+22^{\circ}\text{C}$, AFTER ETHANOL PREEXPOSURE

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Abnormal catecholamine levels have been proposed to be closely related to some psychiatric diseases (e.g. schizophrenia, depressions and others). Many neuropharmacological methods have been commonly used in animal models to examine central aminoergic systems, but due to ethical reasons those methods cannot be applied in humans. Therefore, an important role is ascribed to postmortem determination of brain biogenic amines levels. Results of these studies are valuable for better understanding the pathophysiology of some mental diseases. It is well established that temperature and time of death are the most crucial factors affecting postmortem brain biogenic amine levels. On the other hand, there are few data showing that ethanol given shortly before death can influence their level. Therefore, the aim of the present study was to examine levels of dopamine (DA), serotonin (5-HT) noradrenaline (NA), and their metabolites

(DOPAC, HVA, 3-HT, 5-HIAA) in the rat striatum, kept after death under different conditions. After removal from the skull, brains were kept at three different temperatures: -70°C (dry ice), 4°C (refrigerator) and 22°C for 24 h, to mimic commonly occurring conditions for body preservation. Half of the examined rats were given ethanol (5.0 g/kg *ip*) 2 h before decapitation. Biogenic amines and their metabolites in the tissue extracts were measured using high performance liquid chromatography with electrochemical detection (HPLC/ED).

We have shown that the storage conditions of the samples (temperature) influenced the DA and 5-HT level. Also ethanol modified the 5-HT and DA metabolite levels in the examined tissues. The obtained results, besides the cognitive aspect, could be useful in human necropsy material studies.

The study was supported by Medical University of Silesia.

INFLUENCE OF EPINEPHRINE (NE) INJECTED INTO THE LATERAL GENICULATE NUCLEUS (LGN) ON VISUAL TRANSMISSION (FLASH VISUAL EVOKED POTENTIALS VEPs) IN RATS

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Purpose. Our previous study showed that the activation of opioidergic or dopaminergic receptors had distinctly modified VEP in rats. The aim of present study is to determine the effect of norepi-

nephrine (NE) injected into the lateral geniculate body on VEP.

Method. The experiments were performed on female adult Wistar rats weighing 220–325 g. Un-

der chloral hydrate anesthesia (0.3 ml/100 *ip*) the rats were stereotaxically implanted with electrodes: active electrode under the skull on dura mater in occipital region of the brain and reference one on the skull in the interorbital region. Moreover, guide canula was implanted 2 mm above the planned site of injection into GL. After next 5–7 days the flash visual evoked potentials were recorded by the 1000 LKC electrophysiologically interfaced personal computer system (USA), with Ganzfeld stimulation of both mydriatic eyes (1% mydriacyl and 1% atropine), under the chloral hydrate anesthesia. Every

5 min of the experiment we analyzed VEP curve before and after injection into LGN (in a volume of 1 μ l): 0.9%NaCl and NE at doses of 1, 2.5 and 5 nmol. We have calculated amplitude and latency of negative peak N1 and positive P2 following it.

Results. After NE treatment, the amplitudes of N1 increased up to 130% and amplitudes of P2 increased up to 150% after higher dose only.

Conclusion. The results of this experiment show that NE injected into the lateral geniculate body intensified visual transmission in the rat brain.

INFLUENCE OF INTRACEREBROVENTRICULAR INJECTIONS OF NOREPINEPHRINE ON FLASH VISUAL EVOKED POTENTIALS (FVEP) IN THE RATS PRENATALLY EXPOSED TO SELENIUM

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Purpose. In our previous studies we observed the changed reactivity to various neurotransmitters or neuromodulators in rats prenatally exposed to heavy metals. In this paper, we attempt to demonstrate the effect of prenatal exposure to selenium on FVEP after norepinephrine (NE) given into the lateral brain ventricle.

Method. Pregnant Wistar rats were allowed to drink water with selenium throughout the entire pregnancies until the parturition. Six offsprings of these rats and 6 control rats (the offspring of rats which drank only tap water during pregnancy) were examined when they became four-month-old. Under general anesthesia the rats were implanted with active and reference electrodes (fixed on the dura mater and on the skull respectively) and with the canula into the right lateral brain ventricle. FVEP were obtained before and after injections of

NE at 25 and 50 nmol into the lateral brain ventricle.

Results. There were no differences in mean amplitudes and latencies of N1 and P1 waves between the groups. NE injections caused that the N1 and P1 latencies became longer by about 10–15% in the control group, but they were shortened by about 5–10% in the examined group. The N1 amplitude was increased by about 10–15% in the control group and by about 20–45% in the examined group. The P1 amplitude was decreased by about 20–60% in the control group and was increased by about 30–60% in the examined group.

Conclusion. Prenatal exposure to selenium caused an increase in the sensitivity of visual evoked response after intracerebroventricular injection of NE.

BRAIN [³H]-SCH23390 BINDING AND BEHAVIORAL TRAITS OF APPROACH AND AVOIDANCE DRIVES IN NAIVE RATS

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Dopaminergic transmission has been demonstrated to be essential for a large variety of motor and non-motor functions including approach and avoidance behavior, memory, reward, and sensory perception. However, contribution of the dopamine D₁ receptor family, in different brain structures, to the regulation of various behavioral processes, is not yet clarified enough. The aim of the present study was to examine the correlation of motor and exploratory reactions, and a conditioned fear response, of naive rats with the binding of the dopamine D₁ receptor antagonist [³H]-SCH 23390, in several brain structures visualized with quantitative receptor autoradiography.

A significant positive correlation was found between the ligand binding in the substantia nigra

pars reticulata and both animal motor activity ($r = 0.67, p < 0.05$), and the number of entries into the central sector of the open field ($r = 0.59, p < 0.05$). On the other hand, rat motility and the central entries were negatively correlated with [³H]-SCH 23390 binding within the caudate putamen ($r = -0.64, p < 0.05$ and $r = -0.61, p < 0.05$, respectively). No correlation was revealed between the ligand binding in the examined brain areas and freezing reaction in the contextual fear conditioning test. The present data indicate for the first time a significant, structure dependent, correlation between rat motor behavior and the dopamine D₁ receptor ligand binding, within the nigrostriatal system.

CHOLINERGIC LIGANDS SELECTIVELY MODULATE RAT ANXIETY BEHAVIOR IN THE ANIMAL MODELS OF CONTEXTUAL ANXIETY

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Several reports indicate that selective ligands of the cholinergic receptors may modulate, in a selective way, animal behavior in pre-clinical models of anxiety [1, 3]. There is also evidence that some cholinergic ligands, e.g. nicotine, may act in a different way, dependently on the model of an anxiety reaction [2].

The present study aimed to assess behavioral effects of cholinergic ligands administered intracerebroventricularly (*icv*), in two models of anxiety based on a neophobic reaction, an open field test and conditioned fear test (freezing). For comparative purposes, the benzodiazepine midazolam was applied in the same tests.

Exploratory behavior in the open field test was increased after physostigmine (5 g *icv*) and tacrine (20 g *icv*), while rivastigmine was without effect. Nicotine (20 and 40 g *icv*) and pilocarpine (5 and 25 g *icv*) both increased some parameters of exploratory behavior. Opposite effects were observed after mecamylamine (30 g *icv*) and pirenzepine (10 and 50 g *icv*). Methyllycaconitine and dihydro-erythroidine showed only tendency to decrease exploratory behavior.

In the conditioned freezing test, the drugs were administered immediately before conditioning training; and the time of freezing episodes was evaluated 24 h later. In this test, acetylcholinesterase inhibitors, tacrine and rivastigmine, were without effect. Nicotine dose-dependently decreased time of freezing episodes. Methyllycaconitine acted in a similar way. Midazolam used as a reference compound at the dose of 10 g *icv*

showed a clear-cut tendency to decrease the time of freezing.

In summary, these data provided support for the role of central cholinergic system particularly in the control of anxiety reactions induced by exposition to novel environment.

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INFLUENCE OF ALTERNATING LOW FREQUENCY MAGNETIC FIELDS ON REACTIVITY OF THE CENTRAL DOPAMINE RECEPTORS IN NEONATAL 6-HYDROXYDOPAMINE-TREATED RATS

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The aim of this study was to evaluate the influence of low frequency magnetic field on the reactivity of central dopamine (DA) D₁ receptors in rats with 6-hydroxydopamine (6-OHDA)-induced sympathectomy (animal model of Parkinson's disease). The experiment was carried out on male Wistar rats. On the third day of postnatal life, a lasting and selective chemical damage of central dopaminergic system was induced (chemical sympathectomy) in the rats. The animals received desipramine (20.0 mg/kg *ip*, base form) and after 1 h 6-OHDA HBr (66.7 g *icv*, base form) in a volume of 5 µl of vehicle (0.9% NaCl with 0.1% ascorbic acid) to each

lateral ventricle of the brain (total of 133.4 µg). On the third postnatal day, control animals received desipramine treatment (20.0 mg/kg *ip*, base form) and after 1 h the injection of a volume of 5 µl of vehicle to the lateral ventricles of the brain. Experiments were conducted on adult males. The sympathectomized rats were randomly divided into two groups. Rats from the first group were exposed to alternating, sinusoidal magnetic field with frequency of 10 Hz and induction of 1.8–3.8 mT. Whole body of the animals was exposed to the field for 1 h daily, over the period of 14 days, always at the same time of the day. Sympathectomized rats

from the second group were subjected to sham-exposure, when the applicator connectors received no electric current and therefore the applicator solenoid generated no magnetic field. The control rats were also randomly divided into two groups. The first one was exposed to the field with parameters identical to the above according to the same scheme, and the second group was subjected to sham-exposure. Thus, both sympathectomized and control rats were exposed to alternating magnetic field or sham exposed for 14 days and on day 15 the following evaluations were made: spontaneous irritability, oral activity, and catalepsy.

It was found that in adult rats of Wistar strain with chemically induced sympathectomy, 14 daily exposures to alternating low frequency magnetic field caused a reduction of irritability and oral activity stimulated with SKF 38393 (the agonist of central DA D₁ receptor) and some increase in catalepsy after administration of SCH 23390 (the antagonist of central DA D₁ receptor). The presented investigation indicates that alternating low frequency magnetic fields change the reactivity of central DA D₁ receptor type in rats.

INFLUENCE OF PENTETRAZOLE KINDLING ON THE RAT EMOTIONAL BEHAVIOR

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Interictal behavioral disorders are considered as the symptoms specific for epilepsy and include anxiety and depression. In the present study, we investigated the influence of the multiple, sub-threshold (30 mg/kg *ip*) pentetrazole (PTZ) administration, leading to seizures, on the rat emotional behavior. The animals were considered to be kindled after reaching at least two consecutive stage 4 or 5 seizures. Control animals received the same number of saline injection.

Rats were tested in the open field test, Vogel conflict test, step-down avoidance test, conditioned fear test and ultra-vocalization test (USV). There

were no significant differences between kindled and control rats in the open field, Vogel test and step-down test. However, in the conditioned fear test, kindled rats expressed reduced number of freezing episodes. In the USV test, kindled rats exhibited decreased basal and enhanced shock-induced conditioned vocalization.

This data suggest that PTZ kindling is associated with the behavioral changes in rats, similar to those observed in epileptic patients, and may be an useful model to study the mechanisms contributing to the interictal disturbances in emotional behavior.

INTERACTION OF H₂ RECEPTOR ANTAGONIST, CIMETIDINE, WITH CONVENTIONAL ANTIEPILEPTIC DRUGS IN MICE

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H₂ receptor antagonists are widely used in the patients with gastrointestinal diseases. These drugs have also been reported to induce occasionally convulsions, in conjunction with other symptoms such as confusion, agitation and excitation.

The aim of this study was to evaluate the effects of acute and chronic administration of cimetidine (H₂ antagonist, 3-day and 7-day administration) on the anticonvulsant activity of antiepileptic drugs against maximal electroshock (MES, tonic hind-limb extension taken as the endpoint)-induced convulsions in mice. The following antiepileptic drugs were used: valproate, carbamazepine, diphenylhydantoin and phenobarbital. In addition, the effects of antiepileptic drugs alone or in combination with cimetidine were studied on motor performance. The influence of cimetidine on the plasma levels of the antiepileptic drugs was also evaluated. The experiments were carried out on male mice weighing 20–25 g, housed under standard laboratory conditions.

Cimetidine (up to 200 mg/kg) did not affect the convulsive threshold after acute administration. Nevertheless, cimetidine (20 mg/kg) significantly

increased the anticonvulsant activity of carbamazepine. At 100 mg/kg, cimetidine potentiated both the protective action of carbamazepine and phenobarbital. Valproate and diphenylhydantoin were not affected by cimetidine (up to 200 mg/kg) in this respect. Moreover, cimetidine (20 mg/kg) distinctly reduced the protective efficacy of phenobarbital, for the 3-day and the 7-day treatment. Cimetidine did not alter the protective activity of the remaining antiepileptics after repeated injections. Cimetidine (20 mg/kg), both acutely and repeatedly, did not alter the free plasma levels of valproate, phenobarbital, diphenylhydantoin and carbamazepine, so a pharmacokinetic interaction is not probable. Cimetidine applied with antiepileptic drugs did not impair the performance of mice evaluated in the chimney test.

It may be concluded that the anticonvulsive activity of phenobarbital could be reduced in epileptic patients receiving cimetidine therapy.

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URINE ACTIVITY OF α -HEXOSAMINIDASE (UHEX) AND α -GLUTAMYLTRANSFERASE (UGGT) AS NON-INVASIVE MARKERS OF ALCOHOL ABUSE

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The objective of this study was to assess the value of α -hexosaminidase (uHex) activity and α -glu-

tamyltransferase (uGGT) activity in urine as markers of alcohol abuse, compared to standard serum

markers: the relative amount of carbohydrate deficient transferrin (s%CDT), -glutamyl-transferase (sGGT) and -hexosaminidase (sHex). Areas under Receiver Operating Characteristic curves (ROC AUC) were used as a measure of test performance. It has been demonstrated that in alcohol-dependent persons the uHex test (ROC AUC 0,92) is one of the most powerful discriminating tools, while uGGT (ROC AUC 0,79) has a discriminating power similar to that of sHex (ROC AUC 0,79) but inferior to that of uHex, sGGT and s%CDT (0.92, 0.92 and 0.88, respectively).

In opiate-dependent group, the uHex test (AUC 0.82) is among the best alcohol abuse markers. In

this group the influence of factors other than alcohol is sufficient to render useless the universally used sGGT and sHex tests (AUC 0.65 and 0.69, respectively). For this group of patients, uGGT (AUC 0.73) remains one of three usable tests alongside of uHex and s%CDT.

High discriminative value, low costs, easiness of use and non-invasive character are all features that make uHex and uGGT highly useful tools in the detection of alcohol abuse in alcohol-dependent and opiate-dependent patients.

EFFECTS INDUCED BY SEROTONIN IN GABAergic HIPPOCAMPAL NEURONS

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The hippocampal interneurons are innervated by serotonergic terminals from the raphe nuclei. A number of studies have been conducted to elucidate the effects of serotonin (5-HT) on hippocampal interneurons, however, their results still are conflicting. This is probably due to the great diversity of 5-HT receptor subtypes in the brain. This diversity permits different effects of 5-HT on various neurons and even on the same neuron, as shown by inhibitory 5-HT_{1A} and an excitatory 5-HT₄ receptors co-expressed on the hippocampal pyramidal cells.

In the present study, using intracellular (patch clamp) *ex vivo* recording techniques, we investigated the action of 5-HT on GABAergic interneurons in the rat hippocampal slices.

The obtained results indicated that the population of the studied hippocampal interneurons showed high diversity regarding the effects of 5-HT. About 20% of the recorded cells revealed neuronal hyperpolarization caused by the 5-HT_{1A} receptor activation (the effect was mimicked by 8-OH-DPAT, 5-HT_{1A} receptor agonist). About 20% of interneurons were depolarized by 5-HT and probably this effect was mediated by activation of 5-HT₃ receptors (the effect was partially blocked by zacopride, 5-HT₃ receptor antagonist). Another 30% of the observed neurons showed both responses: first hyperpolarization followed by depolarization. In some cells, 5-HT did not evoke any reaction. The relevance of these effects will be discussed.

CHANGES IN THE EXPRESSION OF GROUP I METABOTROPIC GLUTAMATE RECEPTOR (mGluR) IN ANIMAL MODEL OF DEPRESSION IN THE RAT BRAIN

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Chronic mild stress (CMS) has been reported to be one of the best validated animal models of depression, as it induces behavioral abnormalities that reflect human depression. One of the core symptoms of human major depression is anhedonia, which in stressed rats is measured as a decreased consumption of a 1% sucrose solution and this effect is reversed by imipramine.

In the previous studies, using the CMS procedure, the antidepressant-like effect was observed after chronic treatment with competitive and non-competitive NMDA receptor antagonists (MK-801, ACPC, CGP 37849, D-cycloserine). The studies were designed also to investigate in CMS model the involvement of the NMDA receptor complex in depression. CMS increases the potency of glycine to displace [³H]5,7-DCKA binding to glycine/NMDA sites, and a reduction of the specific [³H]5,7-DCKA binding was observed.

A specific modulation of NMDA responses by mGluR activation has been demonstrated in a num-

ber of brain areas. mGluR1 receptors are co-localized with NMDA receptors in CNS. Recent data have indicated that mGluR ligands may have antidepressant-like properties in animal screening test used to detect antidepressant activity of drugs. It was demonstrated for group I mGluR1 antagonist (MPEP) and group II mGluR agonist (LY354740). Besides, LY354740 possesses antidepressant activity in CMS procedure with the faster onset of action than imipramine.

The aim of our study was to investigate the level of mGluRs in CNS in rats. Using Western blot procedure we showed that mGluR5 receptor protein level was increased in CA1, and decreased in CA3 region of the rat hippocampus in the stressed rats, and imipramine further increased the mGluR5 protein level in CA1 region of the hippocampus.

Our results indicate that mGluR5 can be possibly engaged the mechanism of stress and antidepressant action.

INTERACTION BETWEEN mGluR5 AND NEUROPEPTIDE Y IN THE RAT BRAIN AMYGDALA PARTICIPATES IN THE REGULATION OF ANXIETY

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Neuropeptide Y (NPY) is widely distributed in the neurons of several brain structures and takes part in many functions. In the amygdala NPY-containing neurons and terminals are scattered over the whole nucleus, and the peptide has an anxiolytic action there. Anxiolytic effects have also been ob-

served after the treatment with an antagonist of glutamatergic transmission. Some studies indicated an interaction between glutamatergic system and NPY in the hippocampus. In the present study, the effect of metabotropic glutamate receptor (mGluR5)-antagonist, 2-methyl-6-(phenylethyl)-py-

ridine (MPEP), on NPY expression in the rat amygdala was studied immunohistochemically. Moreover, behavioral studies were performed in which anxiolytic action of MPEP and the effect of the blockade of Y1 receptors in amygdala by the local intraamygdalar injection of BIBO 3304 was investigated in the plus-maze test. In the immunohistochemical studies, MPEP (10 mg/kg, *ip*, 3 times,

every 8 h) caused a decrease in NPY-ir expression in nerve cell bodies and terminals of the amygdala, which may suggest a release of NPY in the structure. In the behavioral studies, MPEP (10 mg/kg) induced an anxiolytic effect which was antagonized by BIBO 3304. This results indicate the involvement of NPY *via* NPYY1 receptors in the anxiolytic action of the mGluR5 antagonist, MPEP.

CARDIOVASCULAR EFFECTS OF (S)-3,5-DHPG, A SELECTIVE AGONIST OF GROUP I METABOTROPIC GLUTAMATE RECEPTORS

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The aim of this work was to assess the action of (S)-3,5-dihydrophenylglycine [(S)-3,5-DHPG], a selective agonist of group I metabotropic glutamate receptors (mGluRs) on arterial blood pressure, the function of isolated rat heart and the contraction of isolated rat aorta. We used the following methods: direct measurement of arterial blood pressure, Langendorff rat heart preparation and perfusion at constant pressure; moreover, rat aortas were cut into rings to measure isometric tension development *in vitro*.

(S)-3,5-DHPG administered at doses of 0.07, 0.2 and 0.6 mg/kg *iv* had no effect on arterial blood pressure during 150 min. (S)-3,5-DHPG (0.01, 0.1

and 1.0 M) had no effect on the contraction but shortened duration of contraction. (S)-3,5-DHPG (0.01, 0.1, 1.0 and 10.0 M) decreased the cardiac contraction amplitude and heart rate (maximal effects at second minute). (S)-3,5-DHPG (0.01, 1.0 and 10.0 M) had no effect on coronary outflow and the compound used at 0.1 M increased coronary outflow by about 20%.

These results may suggest that the activation of group I mGluRs takes part in cardiovascular activity, mainly in heart but the elucidation of the mechanism of action of (S)-3,5-DHPG (direct or indirect) requires further study.

CONTRIBUTION OF CYTOCHROME P-450 ISOENZYMES TO THE METABOLISM OF THE SIMPLEST PHENOTHIAZINE NEUROLEPTIC, PROMAZINE, IN HUMANS. *IN VITRO* STUDIES

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Contribution of human cytochrome P-450 isoenzymes (CYPs) to the promazine N-demethyla-

tion and 5-sulfoxidation was studied in the following *in vitro* models: 1) liver hepatocytes and speci-

fic inducers (rifampicin – CYP3A inducer, TCDD = 2,3,7,8-tetrachlorodibenzo-p-dioxin – CYP1A1/1A2 inducer); 2) liver microsomes: a) correlations between the rate of promazine metabolism and the level of CYPs, b) effect of specific inhibitors on the rate of promazine metabolism (inhibitors: CYP1A2 – naphthoflavone and furafylline, CYP2B6 – metyrapone, CYP2D6 – quinidine, CYP2A6 + CYP2E1 – DDC = diethyldithiocarbamic acid, CYP2C9 – sulfaphenazole, CYP3A4 – ketoconazole); 3) cDNA-expressed human CYPs (1A1, 1A2, 2A6, 2B6, 2C9, 2C19, 2E1, 3A4). The amount of the formed promazine metabolites was assayed using HPLC with UV detection.

The obtained results indicated that the catalysis of N-demethylation and 5-sulfoxidation of promazine in humans was not specific with predominant contribution of CYP1A2 and CYP3A4. Therefore, the pharmacokinetics of promazine should not be easily changed by other drugs. In contrast, the metabolism of promazine in the rat is catalyzed by CYP2D1 and CYP2B2. Thus, one cannot transfer this kind of metabolic data from rats to humans because of different catalytic properties of CYPs in both species.

AGONIST OF GROUP II METABOTROPIC GLUTAMATE RECEPTORS AFFECTS THE REWARDING EFFECTS OF MORPHINE IN THE CONDITIONED PLACE PREFERENCE TEST IN MICE

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LY 354740 is a selective agonist of group II metabotropic glutamate receptors that crosses the blood-brain barrier. We investigated the effects of LY 354740 on acquisition and expression of conditioned place preference (CPP) induced with morphine. Albino Swiss male mice weighing 28–33 g were used for all experiments. The place preference testing apparatus [Stinus et al., *Neuroscience*, 1990, 37, 767–773] consists of 3 rectangular compartments (30 × 14 × 20 cm) accessible from central platform. The 3 compartments differ in distinctive visual, tactile and olfactory cues. The procedure of CPP includes 5 phases: adaptation, pretest, conditioning with morphine, conditioning with saline and posttest. In the experiments on acquisition of the morphine CPP, the animals received the injection of LY 354740 (3 mg/kg *ip*) both 20 min before the injection of morphine (10 mg/kg *ip*) during

the conditioning with morphine and 20 min before the injection of saline on the day of the conditioning with saline. In experiments on expression of the morphine CPP, the mice received injection of LY 354740 (3 mg/kg *ip*) 20 min before posttest session.

Mice conditioned with morphine at 10 mg/kg demonstrate the marked preference for the morphine paired compartment (115.3 s). LY 354740 (3 mg/kg *ip*) inhibits the acquisition (40.67 s) but not expression (118.1 s) of the morphine CPP in mice. LY 354740 (3 mg/kg *ip*) alone does not produce aversive as well as rewarding effect in CPP procedure in mice (7.1 s).

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EFFECTS OF REPEATED ANTIDEPRESSANTS AND 5-HT_{1A} RECEPTOR AGONISTS ON THE SENSITIVITY OF HIPPOCAMPAL CA1 NEURONS TO 5-HT_{1A} RECEPTOR ACTIVATION

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A number of studies indicate that effects on the serotonergic system may underlie the antidepressant and anxiolytic action of different types of medication. In particular, a role of 5-HT_{1A} receptors in depression and anxiety has been suggested. Preclinical and clinical studies with the partial 5-HT_{1A} receptor agonist buspirone indicate its antidepressant and anxiolytic activity. Using extracellular *ex vivo* recording, we studied the changes in the reactivity of rat hippocampal pyramidal CA1 neurons to serotonin and to the 5-HT_{1A} receptor agonist 8-OH-DPAT, evoked by repeated electroconvulsive shock (ECS, for 10 days), imipramine (10 mg/kg *po*, twice daily, for 21 days), 8-OH-DPAT (1 mg/kg *sc*, twice daily for 10 days) and buspirone (2.5 mg/kg *sc*, twice daily for 21 days)

treatments. Activation of 5-HT_{1A} receptors decreased the amplitude of population spikes evoked by stimulation of the Schaffer/collateral-commissural pathway. All the treatments induced an increase in the sensitivity of CA1 cells to activation of 5-HT_{1A} receptors, but antidepressants had much stronger effect. These findings indicate that repeated treatment with 5-HT_{1A} agonists only to some extent mimics the effects of antidepressants on hippocampal 5-HT_{1A} receptors. These differences may be relevant to the antidepressant vs. anxiolytic effects of both types of medications.

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