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## Oral communications

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# Imipramine, moclobemide and fluoxetine inhibit tumor necrosis factor- $\alpha$ release by lipopolysaccharide-activated rat primary mixed glial cells

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Numerous studies suggest that proinflammatory cytokines are involved in pathophysiology of depression and in the mechanism of action of antidepressant drugs.

There are some clinical and experimental data indicating that antidepressants are able to reduce the levels of proinflammatory cytokines. Because the main source of cytokines in the brain are glial cells, the aim of our study was to investigate the influence of some antidepressants on tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) release by lipopolysaccharide (LPS)-activated primary mixed rat glial cultures. Three antidepressants with different mechanism of action on monoaminergic systems: imipramine, moclobemide and fluoxetine were used.

Mixed glial cultures were prepared from cerebral hemispheres of one day old newborn Wistar rats in Dulbecco's Modified Eagle's Medium supplemented with 10% heat-inactivated fetal bovine serum and 1% antibiotic solution. The cells were cultured for 12–14 days (37°C; 5% CO<sub>2</sub>, 95% air, 80% humidity). Because the strongest stimulation of TNF- $\alpha$  release was observed when LPS was administrated at a concentra-

tion of 1  $\mu$ g/ml for 6 h, these parameters were applied in the experiments. Antidepressants were used at concentrations from 10<sup>-8</sup> to 10<sup>-4</sup> M/ml. Only fluoxetine was applied at concentrations up to 10  $\mu$ M because the higher concentration was cytotoxic as determined with the Trypan Blue exclusion method. Levels of TNF- $\alpha$  release were evaluated in culture medium with rat TNF- $\alpha$  ELISA kits (R&D, USA). The intra-assay precision CV for TNF- $\alpha$  was 7.4%. The cultures were stained with *Ricinus communis* agglutinin-1, lectin that binds to the surface glycoproteins on microglia (Vector, USA). The cells were examined using fluorescence microscope (TS-100/F, Nikon). The results of our study have shown that the studied antidepressants inhibit TNF- $\alpha$  secretion by mixed glial cell cultures. Imipramine produced this effect at concentrations from 10<sup>-4</sup> to 100  $\mu$ M, moclobemide from 10<sup>-6</sup> to 10<sup>-4</sup> M and fluoxetine – from 10<sup>-8</sup> to 10<sup>-5</sup> M. The obtained results support the previous observations that antidepressants are able to reduce the levels of proinflammatory cytokines and that TNF- $\alpha$  may be involved in the central mechanism of action of imipramine, moclobemide and fluoxetine.

## The influence of group I mGluR antagonists on the EtOH-induced conditioned place preference in rats

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Glutamate plays an important role in ethanol effects. Antagonists of N-methyl-D-aspartate (NMDA) glutamate receptors may be useful in pharmacotherapy of alcoholism. However, most of these drugs induce numerous side effects, such as fatigue, dizziness or most prominently psychotomimetic effects. Currently, antagonists of group I metabotropic glutamate receptors (mGluRs) are believed to have a milder side-effect profile and have been suggested as potential new therapeutics for alcoholism. Thus, the main aim of the study was to investigate the influence of two selective group I mGluR antagonists, EMQMCM (mGluR1) and MTEP (mGluR5), on the rewarding effect of ethanol measured in the conditioned place preference (CPP) paradigm in rats.

The ethanol-induced CPP procedure (biased design) was described earlier [Kotlińska et al., *Eur J Pharmacol*, 2004] and based on that of Bieńkowski et al. [*Pol J Pharmacol*, 1995]. The procedure consisted of an initial pretreatment of animals ( $n = 8-10$ ) with a single injection of 10% (w/v) ethanol (0.5 g/kg, *ip*) or saline every day for 15 days. During the conditioning phase (8 days), the rats were treated with ethanol (0.5 g/kg, *ip*) or saline (*ip*). Five minutes after the injection, animals were confined to the non-preferred

(white) compartment for 30 min. On alternate days the rats were exposed for 30 min to the preferred (black) compartment after saline injection (*ip*). During the last phase (test day), the guillotine door was removed and the time spent in white (initially non-preferred) compartment was recorded for 15 min.

To investigate the effect of either EMQMCM or MTEP on the expression of ethanol-induced CPP, rats with developed ethanol-induced CPP were pretreated with mGluR antagonists 15 min before placement in the CPP apparatus on the test day. EMQMCM was given at doses of 2.5, 5 and 10 mg/kg, whereas MTEP at doses of 1.25, 2.5 and 5 mg/kg.

The results indicated that EMQMCM (an mGluR1 antagonist) at the doses of 5 and 10 mg/kg decreased the expression of ethanol-induced CPP (5 mg/kg,  $p < 0.01$ ; 10 mg/kg,  $p < 0.001$ ). Similarly, a single injection of MTEP (an mGluR5 antagonist) abolished the expression of place preference at the doses of 2.5 ( $p < 0.05$ ) and 5 mg/kg ( $p < 0.001$ ). Acute injection of both mGluR antagonists to control (saline-treated) animals was without effect on the CPP.

Our data suggest that group I mGluR antagonists could be useful in pharmacotherapy of ethanol addiction to inhibit drug craving and seeking.

## Hypoxia-induced behavioral disturbances in rats can be influenced by LY341495

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LY341495 is a highly potent and selective group II metabotropic glutamate receptor (mGluR<sub>2</sub> and mGluR<sub>3</sub>) antagonist. The mGluR<sub>2/3</sub> mainly has a presynaptic localization and causes a decrease in glutamate re-

lease. Hypoxia enhances excitatory synaptic transmission and induces cognitive deficits. The aim of the study was to investigate the influence of LY341495 on certain behaviors and on the activity of MMP-2

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and MMP-9 in the hippocampus of rats without or after hypoxia. Short-term hypoxia (2% O<sub>2</sub> and 98% N<sub>2</sub>) significantly inhibited locomotor activity, impaired acquisition, consolidation and retrieval of the conditioned responses and spatial acquisition in the water maze, exhibited an anxiogenic effect in the elevated plus maze and increased the activity of the pro-form of MMP-9 in the hippocampus. LY341495 enhanced the locomotor activity of both groups of rats, had no influence on the activity of rats without hypoxia in the elevated plus maze but showed marked anxiolytic effects in rats after hypoxia. It impaired acquisition and retrieval in the passive avoidance but did not influence the consolidation process in this test in rats without hypoxia. However, if used before hypoxia, it im-

proved acquisition and retrieval processes. LY341495 impaired spatial acquisition but did not change reference memory of rats without hypoxia in the water maze test. The same results were observed in rats after hypoxia. Zymograms showed that the activity of both forms, MMP-2 and MMP-9 in the hippocampus of rats without or after hypoxia were elevated after LY341495 administration.

In conclusion, LY341495 had beneficial effects on deficits of behavioral processes induced by hypoxia. This antagonist also rebuilt the extracellular matrix in the hippocampus of rats.

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## Molecular targets for new antidepressant drugs

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Depression and anxiety are serious conditions that often require medical intervention. Although new antidepressant drugs (selective serotonin reuptake inhibitors – SSRIs) have been introduced into clinical practice, not all patients respond to the treatment, and 2–4-week delay of their onset of action, accompanied often by unwanted side effects has been observed. Therefore, a search for new antidepressant and anti-anxiety drugs is still a challenging task. Antidepressant targets and systems dysregulated in depressed or anxious states include the hypothalamic-pituitary-adrenal (HPA) axis, monoaminergic system,  $\gamma$ -aminobutyric acid (GABA) system, and adult hippocampal neurogenesis. Extensive research, concerning different pharmacological systems and targets as well as the understanding of molecular mechanisms underlying pathogenesis of depression may help in

the development of more specific, with shorter onset of action, antidepressant and anti-anxiety drugs.

The aim of our work was to better understand the mechanisms of serotonin transporter functioning and attenuation of acute serotonin autoreceptor response. For that purpose, different compounds having a potential to test SERT and 5-HT<sub>1A</sub> receptor models were synthesized. The compounds exhibited diversified affinity to SERT and 5-HT<sub>1A</sub> receptor and had more or less similar rigid structures in order to occupy similar positions in the SERT and 5-HT<sub>1A</sub> receptor models, respectively. Homology modeling of SERT and 5-HT<sub>1A</sub> receptor proteins has been carried out followed by the examination of ligand-protein interaction. Our work enabled us to identify ligand binding sites at SERT and 5-HT<sub>1A</sub> receptor and to disclose the conformations acquired by the proteins upon ligand binding.

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## Effect of glutamic acid on rat brain endothelial cells cultured in simulated ischemic conditions

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We studied the effect of glutamic acid on the isolated rat brain vascular endothelial cells cultured *in vitro* under ischemic conditions. Moreover, the effect of a metabotropic receptor antagonist (MPEP) on these cells was evaluated. The microvessels were obtained from 14 days old Wistar rats. The cells were seeded on the cell culture plates and maintained at 37°C in Dulbecco's modified Eagle's medium containing 20% fetal bovine serum, antibiotics and bFGF. Conditions of experimental ischemia were obtained by incubation in an atmosphere of 3% O<sub>2</sub>, 92% N<sub>2</sub> and 5% CO<sub>2</sub> in medium without glucose and serum. The brain endothelial cells were identified by immunostaining with a rabbit polyclonal anti-von Willebrand factor antibody.

Cells were exposed to glutamic acid (5000 μM) and MPEP (50 μM). Viability was measured using

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) release test. Oxidative stress was detected with dichlorofluorescein (DCF) and cellular glutathione – with monochlorobimane. Apoptosis was evaluated by Hoechst staining. Glutamate did not affect viability of endothelial cells under normoxia and ischemia over 24 h and 48 h periods and did not change oxidative stress level after 1 h and 3 h exposure periods. Glutathione level was decreased after glutamic acid exposure over 24 h and 48 h in normoxia and ischemia. In conclusion, our experiments showed that glutamic acid did not affect cellular apoptosis under both types of conditions. MPEP (mGluR5 receptors antagonist) had negative effect on endothelial cells during ischemic conditions.

## Effects of postnatal administration of thimerosal on rat development and behavior

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Numerous clinical findings support hypothesis that mercury, which was added to many infant vaccines in the form of thimerosal between 2000–2004, may be one of the factors responsible for autism epidemics currently observed all over the world. Data from Adverse Event Reporting of the Center for Disease Control and Prevention (USA) provide strong epidemiological evidence for a link between vaccine-thimerosal exposure and autism or other neurodevelopmental disorders/diseases. The onset of autistic symptoms in children often follows the administration of vaccine-

thimerosal and symptom emergence is consistent with the expression of developmental mercury toxicity.

In this study, we examined potential neurodevelopmental outcomes following postnatal exposure of rats to thimerosal (Sigma-Aldrich), administered *sc* or *im* from 0.040 mg/kg to 50 mg/kg in four equal doses on days 7–14 after birth. Three strains of rats were used in this experiment: Wistar, Lewis and Brown Norway. Development and behavior of experimental animals was observed. Various behavioral tests were carried out, which evaluated: open field locomotor and ex-

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ploratory activity, motor coordination, pain reaction (hot plate), learning and memory (water maze), prepulse inhibition, sociability (social interaction test). Growth of animals was monitored and after animal sacrifice, weight of brains was measured.

Thimerosal had variable, often biphasic, effects on different measured behaviors, which were strain- and dose-dependent, but no dramatic behavioral impairments were observed at doses tested. Data will be discussed in the context of rodent model of autism following postnatal exposure to mercury.

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## Phenotypic characterization of the Warsaw High Preferring (WHP) and Warsaw Low Preferring (WLP) lines of rats selectively bred for high and low alcohol consumption

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The Warsaw High Preferring (WHP) and Warsaw Low Preferring (WLP) lines were bred from Wistar foundation stock to obtain lines of rats that differ in their intake and preference for ethanol (EtOH) solutions. The WHP line has met several major criteria for an animal model of alcoholism. The WHP rats voluntarily drink excessive amounts of EtOH while the WLP rats consume very low or negligible amounts of EtOH. The patterns of EtOH consumption in WHP and WLP lines are stable in time and independent of the manner of access to EtOH solutions. Notably, when exposed to the increasing EtOH concentrations WHP rats gradually increased total EtOH intake while the WLP rats consumed invariably very low amounts of EtOH. Furthermore, the WHP rats but not WLP rats show an increased responsiveness to the locomotor stimulatory effects of low dose of EtOH. The op-

erant procedure of oral self-administration of EtOH, i.e. "work for alcohol", has been considered a reliable measure of the reinforcing effect of EtOH. The rats from WHP and WLP lines of 32nd generation were tested for operant responding for EtOH (oral self-administration). The results demonstrate that WHP and WLP rats similarly acquired and maintained lever pressing reinforced by EtOH under FR1 and FR2 schedules of partial reinforcement. On the other hand, only WHP rats displayed the high ability to acquire and maintain lever pressing for EtOH under FR3 procedure. This result suggests that EtOH may act as a stronger reinforcer in WHP rats than in WLP rats.

The result of our most recent study shows that the cannabinoid CB<sub>1</sub> receptor antagonist SR-141716) 2.5–10 mg/kg *ip*) significantly reduced EtOH intake in WHP rats.

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## Differences in [<sup>3</sup>H]CGP 54626 binding to GABA<sub>B</sub> receptors in the rat brain during cocaine self-administration, its withdrawal and relapse

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There is a considerable evidence from animal models and human neuroimaging studies that chronic exposure to cocaine changes release and turnover of the inhibitory neurotransmitter  $\gamma$ -aminobutyric acid (GABA) in the brain [Jung et al., *Synapse*, 1999; Ke et al., *Psychiatry Res*, 2004]. Moreover, a chronic administration of cocaine attenuates the GABA<sub>B</sub> receptor function in the prefrontal cortex and ventral tegmental area [Jayaram and Steketee, *J Neurochem*, 2004; Kushner and Unterwald, *Life Sci*, 2001]. Till now, there has been no reports showing if voluntary (active) administration of cocaine affects GABA<sub>B</sub> receptors in the rat brain and whether the changes are due to direct pharmacological actions of cocaine or are related to motivational states dependent on active drug-seeking behavior.

In the present study, we used *in vitro* autoradiography to investigate if cocaine self-administration, its withdrawal and the relapse induce changes in [<sup>3</sup>H]CGP 54626, a GABA<sub>B</sub> receptor antagonist, binding to GABA<sub>B</sub> receptors in a variety of brain regions in rats. We used a “yoked” procedure in which rats were tested simultaneously in groups of three, with only one rat actively self-administering cocaine while the other two received yoked injections of either cocaine or saline. Male Wistar rats were trained to intravenously self-administer cocaine (0.5 mg/kg/infusion) under a fixed ratio 5 schedule of reinforcement; each cocaine infusion was associated with presentation of a cue (light + tone). Following a stabilized cocaine self-administration, the rats underwent the 10-day extinction (cocaine was replaced by saline) and reinstatement tests induced by either cocaine (10 mg/kg, *ip*) or the cue. The animals were sacrificed after the

last maintenance or withdrawal sessions or following the reinstatement test.

We found a significant (ca. 20%) decrease in [<sup>3</sup>H]CGP 54626 binding in the nucleus accumbens in rats actively and passively administered cocaine. Moreover, only passively administered cocaine produced a decrease in the binding in the frontal cortex and prefrontal cortex, septum and dorsal striatum. Following 10-day withdrawal in a group of rats previously actively self-administering cocaine, a 20–40% decrease in [<sup>3</sup>H]CGP 54626 binding to GABA<sub>B</sub> receptors was found in almost all investigated brain areas, except for the paraventricular thalamic nucleus where a 25% increase was shown. The cocaine (10 mg/kg, *ip*)-induced reinstatement of responding in rats actively administering cocaine produced a 10–30% increase in [<sup>3</sup>H]CGP 54626 binding in the frontal and cortex, prefrontal cortex, septum, dorsal striatum and the core of the nucleus accumbens. The reinstatement of responding which was induced by the cue previously paired with cocaine self-administration induced a decrease in GABA<sub>B</sub> receptor level in the laterodorsal thalamic nucleus and amygdaloid nucleus.

Summarizing, decreases in [<sup>3</sup>H]CGP 54626 binding to GABA<sub>B</sub> receptors during cocaine self-administration are probably related to the effects of cocaine *per se* and not to the motivated process of reinforced responding. The reduction in the GABA<sub>B</sub> receptor binding following cocaine withdrawal and the cue-induced reinstatement or the increase in binding after an cocaine exposure (*ip*) may be linked to neuroadaptive changes due to previous active administration of cocaine.

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## Effect of FK506 and cyclosporine A on the expression of BDNF, tyrosine kinase B (TrkB) and p75 neurotrophin (p75<sup>NTR</sup>) receptors in astrocytes exposed to simulated ischemia *in vitro*

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We investigated whether the immunosuppressive drugs (FK506 and cyclosporine A) might increase brain-derived neurotrophic factor (BDNF) protein and/or mRNA expression in ischemic astrocytes and whether this increase might be related to changes in the nuclear of expression p-CREB, p-Erk1/2 and p-Akt. We also determined the influence of these immunosuppressants on protein and mRNA levels of TrkB and p75NTR receptors. On the 21st day *in vitro*, cultures of rat astrocytes were subjected to ischemic conditions simulated *in vitro* (combined oxygen glucose deprivation, OGD) for 8 h and exposed to FK506 (10–1000 nM) and cyclosporine A (0.25–10 μM). We

demonstrated that FK506 and cyclosporine A (at concentration of 1 μM and 0.25 μM, respectively) stimulated the expression and release of BDNF in cultured rat cortical astrocytes exposed to OGD. We have also shown that immunosuppressants at those doses simultaneously caused the increase in p-CREB and p-Erk1/2 expression in astrocyte nuclear fraction. Furthermore, the obtained results of RT-PCR and Western blot analysis provided the evidences of a modulating influence of these drugs on the expression of trkB and p75NTR genes and their protein products in ischemic astrocytes.

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## Involvement of serotonin (5-HT)<sub>1B</sub> receptors in the depressive-like behavior in rats withdrawn from cocaine self-administration

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It is well documented that withdrawal from cocaine in humans induces symptoms that appear comparable to those of a major depressive disorder. In preclinical studies, discontinuation of repeated treatment with an abused drug induces depressive-like behavioral changes, i.e. increases in immobility time in the forced swim test (FST) [Barr and Markou, *Neurosci Biobehav Rev*, 2005]. There is a lot of evidence for a role of serotonin (5-HT) neurotransmission in the psychopathology of depression and in the mechanism of action of antidepressant drugs [Leonard, *Drugs Today*, 2007]. There are also studies investigating involvement of

5-HT<sub>1B</sub> receptors in this disorder [Tatarczyńska et al., *Eur J Pharmacol*, 2004, 2005].

In the present study, we investigated the role of 5-HT<sub>1B</sub> receptors and their pharmacological stimulation on immobility, swimming and climbing behavior in the modified FST in either naive male Wistar rats or animals withdrawn from cocaine self-administration. To this end, we used the selective 5-HT<sub>1B</sub> receptor antagonist SB 216641 and the agonist CP 94253.

When given to naive rats, SB 216641 (2.5–7.5 mg/kg, *ip*) and CP 94253 (0.625–2.5 mg/kg, *ip*) dose-dependently decreased immobility time in the FST.

Both investigated drugs increased swimming and did not affect climbing behavior.

In the second part of our experiment, rats were trained to self-administer cocaine (0.5 mg/kg/infusion) under a fixed ratio 5 schedule of reinforcement in 2-h daily sessions (Monday–Saturday) and then withdrawn from cocaine. In those rats, we observed a significant increase (by 40%) in immobility time and a tendency to decrease climbing on the 3rd extinction day. SB 216641 (2.5–5 mg/kg, *ip*) presented a tendency to decrease immobility time, while its dose of 5 mg/kg significantly increased swimming and climbing behaviors. Rats withdrawn from cocaine self-administration showed significant reduction of

immobility time following CP 94253 at 1.25 mg/kg and a tendency to increase climbing behavior.

Our results indicate that 5-HT<sub>1B</sub> receptor antagonist and agonist produce in naive rats effects that are characteristic of antidepressant drugs. Moreover, the activation of these receptors by using a selective agonist seems to counteract the depressive-like effect in rats withdrawn from cocaine self-administration. These findings may suggest the therapeutic potential of 5-HT<sub>1B</sub> receptor agonists for the treatment of cocaine withdrawal symptoms.

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## Memantine attenuates neuronal apoptosis in primary cerebellar granule cell cultures: stimuli- and development-dependent effect

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Memantine is an NMDA receptor antagonist clinically used in the treatment of moderate to severe Alzheimer's disease. Its neuroprotective properties were shown in several experimental models of neuronal cell injury. Moreover, its beneficial action was also shown in attenuation of neuronal apoptosis which was connected with overstimulation of NMDA receptors. In the present study, we evaluated the impact of memantine on apoptotic processes evoked by stimuli which did not engage NMDA receptor in their cell damaging effects. We used primary cerebellar granule cell (CGC) cultures at 7 and 12 day *in vitro* (DIV) and cell death was induced by agents acting *via* mitochondrial (staurosporine, low potassium) or death receptor (doxorubicin) pathway of apoptosis. All tested pro-apoptotic agents induced cell death in CGC with higher cell-damaging effects in 7 DIV neurons. Memantine (0.1–2 μM) partially attenuated the staurosporine (0.5 μM)- and doxorubicin (0.5 μM)-induced apoptosis in CGC only in 7 DIV cells as evidenced by

attenuation of toxin-evoked DNA fragmentation and LDH release, but not caspase-3 activity. In low potassium(LP)-induced apoptosis model, we observed a beneficial effect of memantine on LP-evoked LDH release and DNA fragmentation, but not on caspase-3 activity in both, 7 and 12 DIV cerebellar granule cells. Moreover, in all tested models of apoptotic cell death in CGC there was no beneficial effect of other NMDA antagonists, a competitive, AP-5 (100 μM) and uncompetitive, MK-801, (1 μM) one. These data showed that the antiapoptotic effects of memantine in different models of neuronal cell damage were developmentally regulated and suggested existence of other mechanisms, apart from NMDAR, for its neuroprotective action.

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# The effect of thyrotropin-releasing hormone (TRH) and its analogues on staurosporine-induced toxicity in differentiated human neuroblastoma SH-SY5Y cells

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Prevention of neuronal damage in neurodegenerative diseases is an important and still unresolved problem of the present medicine. Despite numerous studies, no neuroprotective drug has been found. It seems that substances which act through various mechanisms to preserve viability of neurons will be more promising as neuroprotective drugs. Among agents promoting cell survival, neuropeptides, especially thyrotropin-releasing hormone (TRH; pGlu-His-Pro-NH<sub>2</sub>) which is a modulator of CNS deserves our attention. TRH is known to exert neuroprotective effects *in vitro* and *in vivo*, however, its potential utility is limited due to its rapid metabolism. The aim of the present study was to estimate the effects of TRH and its analogues RGH-2202 (L-6-keto-piperidine-2-carbonyl-L-leucyl-L-prolinamide), Z-TRH (Z-pGlutamyl-Histydyl-Pro-linamide) and CG-3703 (Montirelin) on staurosporine-induced cytotoxicity in differentiated with retinoic acid (RA, 10 μM for 7 days) human neuroblastoma (SH-SY5Y<sub>diff.RA</sub>) cells.

Exposure of SH-SY5Y<sub>diff.RA</sub> cells to 0.5 μM staurosporine (a nonselective inhibitor of protein kinases)

for 24 h resulted in a significant increase in lactate dehydrogenase (LDH) activity and a decrease in cell viability as verified by MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl-tetrazolium bromide)-staining assay. In order to better estimate the effect of TRH and its analogues on staurosporine-induced apoptosis, caspase-3 activity was also determined. Pretreatment of SH-SY5Y<sub>diff.RA</sub> cells with TRH or its analogues at a concentration of 0.1–50 μM at 48 h alleviated staurosporine-induced toxicity. Furthermore, the peptides under study differed with respect to their protective activity against staurosporine-induced apoptosis. CG-3703 was the most effective in dose-dependent attenuation of caspase-3 activity, whereas TRH was most effective at its lower concentration.

These data confirm hypothesis on neuroprotective potential of TRH and its analogues.

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## Effect of chronically administrated SSRIs on kynurenic acid – an *in vivo* study

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Kynurenic acid (KYNA) is the only known endogenous antagonist of excitatory amino acid receptors and of α7 nicotinic receptors. Brain KYNA displays

high affinity for the glycine site of N-methyl-D-aspartate (NMDA) receptor complex. The cerebral synthesis of KYNA from its bioprecursor L-kynure-

nine is catalyzed by two distinct kynurenine aminotransferases (KAT I and KAT II). The disturbances of KYNA production have been linked to epilepsy, Huntington's disease, Alzheimer's disease, schizophrenia, AIDS-related dementia and other disorders. Recent experimental data have indicated that NMDA receptor antagonists display antidepressant-like activity in preclinical models. It was also demonstrated that chronic antidepressant therapy changed the function of NMDA receptor complex. The aim of this study was to investigate the effect of chronic administration of selective serotonin reuptake inhibitors (SSRIs): citalopram and fluoxetine on the brain formation of KYNA in rats.

The animals were given citalopram or fluoxetine (10 mg/kg, *ip*) for 1 or 14 days. Brain structures (cortex, hippocampus, striatum) were collected 24 h after the last injection of the drug, and the level of KYNA and KATs activities were assessed. KYNA was quantified using HPLC system with fluorometric detector.

A single administration of citalopram and fluoxetine affected neither KYNA levels nor KATs activities in

all studied brain structures. Chronic administration of citalopram increased KYNA level in the hippocampus, but not in the cortex or striatum, up to 135% ( $p < 0.05$ ) of control. Chronic treatment of fluoxetine increased KYNA level both in the cortex and hippocampus, up to 130% ( $p < 0.05$ ) and 152% ( $p < 0.001$ ), respectively. Citalopram enhanced the KAT I and KAT II activity in the hippocampus up to 129% ( $p < 0.05$ ) and 131% ( $p < 0.05$ ) of control, respectively. It also increased KAT II activity in the cortex, up to 129% ( $p < 0.05$ ). Fluoxetine increased KAT I and KAT II activity both in the hippocampus and cortex, up to 142% ( $p < 0.01$ ), 161% ( $p < 0.001$ ) and up to 120% ( $p < 0.05$ ), 125% ( $p < 0.05$ ), respectively. The data presented here indicate that chronic SSRI therapy might increase cortical and hippocampal level of KYNA and thus suggests a novel mechanism of action of SSRIs.

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## The effect of cortical serotonin depletion in rats, differing in their sensitivity to pain, on the fear response to the aversive context, GABA levels, and c-Fos expression

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We have explored differences in animal reactivity to conditioned aversive stimuli using the conditioned fear test (a contextual fear – freezing reaction), in rats subjected to the selective 5,7-DHT lesion of the prefrontal cortex serotonergic innervation (M2 cortical area) and differing in their response to the acute painful stimulation, a foot-shock (HS – high sensitivity, and LS – low sensitivity, selected arbitrarily according to their behavior in the 'flinch-jump' pre-test). In HS rats, the serotonergic lesion significantly disinhib-

ited rat behavior controlled by fear, enhanced c-Fos expression in the M2 prefrontal area, and increased the concentration of GABA in the basolateral amygdala, measured *in vivo* after the testing session of the conditioned fear test. The LS animals revealed an opposite pattern of behavioral and biochemical changes after serotonergic lesion, viz. an increase in the duration of freezing reaction, and expression of c-Fos in the basolateral amygdala, and a lower GABA concentration in the basolateral amygdala. In control condi-

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tions, c-Fos expression did not differ in LS, HS and naive, non-conditioned and non-exposed to the test cage animals. The present study adds more arguments for the control role of the prefrontal cortex serotonin in processing of emotional input by other brain centers.

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Moreover, they provide experimental data, which may help to better explain the anatomical and biochemical basis of differences in individual reactivity to stressful stimulation, and, possibly, to anxiolytic drugs with serotonergic or GABAergic profiles of action.

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## Neurosteroids attenuate staurosporine- and glutamate-induced cell damage in neurons

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Neuroprotective action of some neurosteroids was shown in several *in vitro* and *in vivo* studies, but their interaction with apoptotic/necrotic processes has been only partially unraveled. The aim of the present study was to examine the effect of dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), pregnenolone (PGL) and allopregnanolone (Allo) on staurosporine-, glutamate-, and NMDA-induced damage in primary cortical neuronal culture. DHEA, DHEAS and PGL (0.1 and 1  $\mu$ M) attenuated the staurosporine-evoked lactate dehydrogenase (LDH) release and decreased the number of apoptotic cells as shown by Hoechst's staining, whereas Allo was without effect. The neuroprotective effect of neurosteroids was accompanied neither with attenuation of staurosporine-induced caspase-3 activity nor by this toxin-induced decrease in mitochondrial membrane potential. It was also shown that protective effects of DHEA and PGL against staurosporine-induced cell damage were partially inhibited by extracellular sig-

nal-regulated kinase (ERK) – mitogen-activated protein kinase (MAPK) inhibitor – PD 98059 (5  $\mu$ M), but not by phosphatidylinositol-3-kinase (PI3-K) inhibitors such as LY 294002 (1  $\mu$ M) or wortmannin (10 nM). Further study demonstrated that glutamate-, but not NMDA-induced cell damage was attenuated by DHEA, DHEAS, and PGL. The results of the present study suggest that excitatory neurosteroids DHEA, DHEAS and PGL at physiological concentrations participate in the inhibition of cortical neuronal degeneration elicited by staurosporine and glutamate, whereas the most potent positive modulator of GABA<sub>A</sub> receptor – Allo – has no effect. Moreover, neurosteroids appear to attenuate the staurosporine-induced cell damage in a caspase-3 independent way and their neuroprotective mechanism of action involves the increase in ERK-MAPK phosphorylation.

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## Is kynurenic acid engaged in the development of kindling of seizures?

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Kynurenic acid (KYNA) is an endogenous brain compound that inhibits the activity of ionotropic excitatory amino acid receptors. The potential role of altered KYNA-mediated modulation of EAA receptors in the human neuropathology has been postulated [Dichter and Alaya, *Science*, 1987]. In particular, the disturbances in KYNA production have been linked to the occurrence of epilepsy and other neurological disorders [Nemeth et al., *Neuropharmacology*, 2004]. In epileptic children with West syndrome, decreased KYNA level in the cerebrospinal fluid was reported suggesting that seizures may be related to the disturbances in KYNA metabolism [Yamamoto et al., *Pediatr Neurol*, 1994]. The available experimental data and theoretical considerations have suggested that KYNA as an EAA receptor antagonist, could have therapeutic effects in a number of neurological disorders. Kindling is a process where the repeated administration of subconvulsant electrical stimulation of the amygdala or administration of subthreshold doses of proconvulsant agents leads to generalized convulsions [Morimoto et al., *Prog Neurobiol*, 2004]. In the present study, we have determined the levels of endogenous kynurenic acid in the selected brain regions of

pentetrazole (PTZ) kindled rats (hippocampus, amygdala CPU, nucleus accumbens, prefrontal, piriform, entorhinal cortex). The seizure stage was scored according to the Racine's five-stage (1–5) scale. PTZ administration was continued until different seizure stage was obtained, ranging from the stage 1 to the stage 5 of seizures. After achieving the appropriate seizure stage, the animals were sacrificed followed by HPLC analysis of KYNA concentration in the brain structures.

The significant decrease in KYNA level in the hippocampus, amygdala and prefrontal cortex was observed in the fully kindled animals (stage of seizures 4 and 5). There were no changes in the KYNA concentration between fully kindled and control animals in the entorhinal and piriform cortex.

The regionally selective alternations in KYNA concentration in the hippocampus and amygdala indicate that these areas may be critically involved in seizure propagation in the kindled model of temporal lobe epilepsy. The decreased levels of an endogenous glutamate antagonist KYNA in the hippocampus, amygdala and prefrontal cortex may be the cause of an enhanced excitability of these brain regions.

## Antidepressant effects of venlafaxine and nicotine in rats – behavioral and receptor studies

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References have indicated that the effect of venlafaxine (VEN) is related to increased both serotonergic and adrenergic neurotransmission in the central nervous system (CNS).

Antidepressant activity of pure nicotine (NIC) depends for instance on its effect on the central nicotinic receptors and is associated with its ability to release such neurotransmitters as: serotonin (5-HT), noradre-

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naline (NA), dopamine (DA). For drugs to have antidepressant activity, plastic changes in the brain, called  $\beta$ -down-regulation, are required [Vetulani and Sulser, Nature, 1975]. Fast antidepressant effect of VEN may also be the result of increased concentration of NA and 5-HT in the brain due to inhibition of their reuptake by VEN.

The studies were conducted to assess the antidepressant activity in rats following a single and repeated administration of VEN or NIC, and the effect of their combined administration on the density of  $\beta$ -adrenergic receptors in the brain cortex.

In the experiment, female Wistar rats weighing 200–220 g were used. VEN had been administered at the dose of 20 mg/kg *po*, and NIC at 0.2 mg/kg *sc* b.i.d. for 14 days. Antidepressant activity was evaluated in the Porsolt's test. To perform receptor studies, the brain cortex was sampled from rats following decapitation. Radioligand [<sup>3</sup>H]CGP12177 was used and propranolol served as a displacer. Scatchard analysis determined the  $B_{max}$  (density) value and  $K_d$  (affinity) of  $\beta$ -adrenergic receptors in rat brain tissue [Popik et al., Eur J Pharmacol, 2005].

VEN and NIC both had antidepressant effect as early as after a single administration. Combined administration of VEN and NIC increased antidepressant activity of VEN. Upon 14 days of administration of VEN or NIC, no changes in  $\beta$ -receptor density in the rat brain tissue were reported. Following the combined administration of VEN and NIC, a statistically significant decrease in  $\beta$ -receptor density in the rat brain tissue was reported. No changes were found, however, in  $\beta$ -receptor affinity.

The absence of  $\beta$ -down-regulation following VEN administration may be explained by the serotonergic component of the drug which masks the occurrence of  $\beta$ -receptor desensitization induced by the NAergic mechanism. NIC has no direct effect *via*  $\beta$ -adrenergic receptors, so it may be assumed that NIC ability to induce  $\beta$ -down-regulation following its combined administration with VEN is a result of increased NA availability due to enhanced release of NA by NIC and inhibition of its reuptake by VEN.

In conclusion, it may be assumed that nicotine enhances antidepressant activity of VEN, and the process is associated with plastic changes occurring in the brain.

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## Chemotherapy of acute lymphoblastic leukemia in children: the changes in glutamate and aspartate concentration in cerebrospinal fluid during treatment

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In the past 25 years, major advances in the treatment of childhood acute lymphoblastic leukemia (ALL) have increased event-free survival to greater than 70%. However, post-treatment neurotoxicity is still a problem. The aim of this study was to find out if the changes in excitatory amino acid (EAA) concentrations in cerebrospinal fluid (CSF) contribute to the neurotoxicity of standard treatment protocols.

Eighteen ALL patients without neurological symptoms or an identified central nervous system (CNS) disease were examined seven times: once at the initia-

tion of the treatment, five times during the consolidation phase and finally before the maintenance therapy.

The mean CSF glutamate and aspartate levels at the diagnosis were  $5.07 \pm 2.65$   $\mu\text{mol/l}$  and  $4.55 \pm 3.18$   $\mu\text{mol/l}$ , respectively, and showed no correlation with initial leukocytosis, organomegaly and lactate dehydrogenase concentration. Dynamic analysis of these levels revealed a statistically significant ( $p < 0.05$ , both) increase in glutamate and aspartate on the 59th day of the treatment when the levels were  $8.17 \pm 2.97$  and  $15.01 \pm 11.05$ , respectively. Also, there was a sig-

nificant increase in glutamate at one point during consolidation phase ( $8.57 \pm 5.16$ ;  $p < 0.05$ ). The changes in both examined EAA were strongly positively correlated during the treatment period ( $r = 0.75$ ;  $p < 0.05$ ).

Standard ALL treatment increases EAA levels which, in some cases, may contribute to its neurotoxicity. Early changes in EAA levels during the treatment may point to the particular CNS susceptibility to the drug-induced impairment in some patients.

## Antidepressant drugs inhibit an increase in the brain protein phosphatase 2A concentration in prenatally stressed rats

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An increasing body of evidence indicates that glucocorticoids are involved in the pathogenesis of depression and that antidepressant drugs inhibit hypothalamic-pituitary-adrenocortical (HPA) axis activity and some effects exerted by glucocorticoids. Recent reports implicate that intracellular signal transduction pathways, regulated especially by protein kinases and phosphatases, may be an important target of antidepressant drug action. It has been shown that these drugs activate many protein kinases, however, their influence on protein phosphatases is poorly recognized. Since the inhibition of protein phosphorylation through activation of phosphatases is a well-known mechanism of glucocorticoid action, the aim of the present study was to find out if in prenatally stressed rats, a well-characterized animal model of depression, the level of protein phosphatase 2A (PP2A) is changed and whether antidepressant drugs inhibit these changes.

Pregnant Sprague-Dawley rats were subjected daily to three stress sessions (from day 14 of pregnancy until delivery). At 3 months of age, control and prenatally stressed male rats were injected once daily with 0.9% saline, imipramine hydrochloride (10 mg/kg), fluoxetine hydrochloride (10 mg/kg), mirtazapine (10 mg/kg) or tianeptine (10 mg/kg) for 3 weeks. Next, in order to verify the used model, the animals were tested for behavioral (Porsolt test, open field test) and endocrine (corticosterone level) changes. The rats were killed 24 h after the last dose of antide-

pressant drugs and the concentration of the PP2A was determined in the hippocampus and prefrontal cortex by Western blot method.

It was found that prenatally stressed rats displayed a prolonged immobility behavior in the forced swimming test and spent a shorter time in the illuminated place in the open field test, i.e. they showed depression- and anxiogenic-like behavior. Also corticosterone concentration 1 h after acute stress was higher in prenatally stressed rats than in control animals. Treatment with imipramine, fluoxetine, mirtazapine or tianeptine decreased all these stress-induced changes. Western blot study showed that prenatally stressed animals had higher PP2A level in the hippocampus and frontal cortex and that all four antidepressants under study attenuated these alterations.

PP2A, a major serine/threonine phosphatase, is known to enhance serotonin transporter function, inhibit tyrosine hydroxylase activity and attenuate protein kinase A (PKA), PKB $\alpha$ , PKC $\alpha$  and mitogen-activated protein kinase, i.e. it causes changes characteristic of depression. The obtained results suggest that the increase in the brain PP2A concentration might be at least one of the mechanisms underlying depression induced by prenatal stress and that this phosphatase can be an important target of antidepressant drug action.

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## Hippocampal amino-acid profile in the kindled rats: a microdialysis study

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It is well known that epileptic seizures can be produced by an imbalance between the processes of neuronal excitation and inhibition with glutamate, GABA, aspartate, glycine and taurine playing an important roles [Meldrum et al., *Epilepsy Res*, 1999]. In this context, it has been hypothesized that seizures and epileptogenesis may be due to overactivation of excitatory pathways, utilizing glutamate or other excitatory amino acids, and/or a lesser activity of inhibitory pathways utilizing GABA and other inhibitory neurotransmitters [Baran, *Amino Acids*, 2006; Dichter and Ayala, *Science*, 1987; Sato et al., *Electroencephalogr Clin Neurophysiol*, 1990]. There is some evidence that brain amino acid concentrations may be altered in epilepsy [Carlson et al., *Neurosci Lett*, 1992; Wilson et al., *Epilepsy Res*, 1996]. However, the relationship between changes in the local concentration of amino acids in different brain structures and seizures is not well recognized. Brain limbic nuclei (e.g. the amygdala and hippocampus) have been most frequently indicated to be important in the recruitment of kindling of seizures [Cohen et al., *Science*, 2002]. It has also been shown that the hippocampus has the lowest seizure threshold [Morimoto et al., *Electroencephalogr Clin Neurophysiol*, 2004]. Moreover, hippocampal sclerosis, represented by cell loss in the CA1 and CA3 region and in the dentate gyrus, is the most often en-

countered pathological finding in temporal lobe epilepsy patients [Blumcke et al., *J Comp Neurol*, 1999; Cavazos and Sutula, *Brain Res*, 1990].

Considering the important role of hippocampal formation in the kindling phenomenon, in the present study we examined, by microdialysis, hippocampal steady state levels of extracellular amino acids (glutamate, glycine, GABA, aspartate, alanine, taurine, arginine, glutamine, histidine) in pentetrazole-kindled and freely moving rats. It was found that in the kindled animals, the concentration of alanine, arginine, glutamate, aspartate and taurine was increased in the interictal period of seizures compared to the control group, whereas kindling reduced the extracellular levels of GABA. No differences between kindled and not-kindled animals in the glycine, histidine and glutamine levels were present. There also appeared an over 4-fold increase in the Glu/GABA ratio, a theoretical marker of the neuronal excitation level, in the kindled animals. A multivariate classification tree analysis showed that the hippocampal concentration of taurine, together with GABA and Glu, had relatively the largest prediction accuracy in discriminating between kindled and non-kindled animals, suggesting a specific role of these amino acids in the shaping of a new equilibrium between excitatory and inhibitory processes in the hippocampus of kindled animals.

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## Anticonvulsant and adverse effect profiles of the H1 receptor antagonist, cetirizine, in an experimental model of tonic-clonic seizures and chimney test in mice

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Histamine is one of the aminergic neurotransmitters, playing an important role in the regulation of several physiological processes. There are several subtypes of histamine receptors: H1, H2, H3, and H4. H1 receptor antagonists, including classical antiallergic drugs, occasionally induce convulsions in children and patients with epilepsy. Previously, it was reported in preclinical experiments that astemizole (an H1 receptor antagonist) diminished the anticonvulsant properties of commonly used antiepileptic drugs.

The aim of this study was to evaluate the effects of cetirizine, a newer antagonist of H1 receptors, administered at a single dose and for 7 days, on the anticonvulsant activity of antiepileptic drugs against maximal electroshock (MES)-induced seizures in mice. The tonic hindlimb extension was taken as the endpoint. The following antiepileptic drugs were used: valproate, carbamazepine, diphenylhydantoin and phenobarbital. The experiments were carried out on male

mice weighing 20–25 g. Cetirizine (administered singly or for 7 days at a dose of 10 mg/kg) reduced the threshold for electroconvulsions, being simultaneously without effect upon this parameter at lower doses. Cetirizine (5 mg/kg) did not alter the protective effect of antiepileptic drugs in the MES test (after 1 and 7 days of treatment). Cetirizine (10 mg/kg, administered singly) also remained ineffective in this respect. However, cetirizine (10 mg/kg, administered chronically for 7 days) considerably reduced the protective efficacy of phenobarbital and carbamazepine against electroconvulsions, by increasing their ED<sub>50</sub> values from 19.1 to 35.6 mg/kg, and 9.4 to 17.2 mg/kg, respectively. Cetirizine (10 mg/kg) did not affect the protective activity of valproate and diphenylhydantoin.

Bearing in mind that second-generation H1 receptor antagonists are usually administered for a long time, the results of this study indicated that cetirizine should be used with caution in epileptic patients.

## Comparison of methadone and morphine potential for sensitization to methadone in the rat

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Harm reduction by methadone substitution therapy in opiate addicts is undisputable [Kreek and Vocci, *J Subst Abuse Treat*, 2002]. However, the curative power of this method has been questioned, and addictive potential of methadone raises fears among clini-

cians [Mas-Nieto et al., *Br J Pharmacol*, 2001]. We have earlier studied the effects of morphine challenge on locomotor activity (LA) and brain regional Fos expression in rats given various morphine or methadone pretreatments. That study revealed sensitization of LA

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response in daily morphine-, but not in daily methadone-pretreated rats, and substantial differences between the respective regional Fos protein response patterns. In the present study, we compared the effects of *sc* methadone hydrochloride (1 or 2 mg/kg/day) and *sc* morphine sulfate (10 mg/kg/day) pretreatments (6 doses/week, 14 doses in total, followed by a 2-week withdrawal) on LA (61–85 min post-injection) and regional Fos protein expression (2 h post-injection) responses to *sc* methadone challenge (1 mg/kg). Significant sensitization of the LA response was found only in the morphine-pretreated rats. Analysis of Fos protein expression in the selected brain regions (paraven-

tricular thalamic nucleus, nucleus accumbens, striatum and cingulate and motor cortices) showed considerably stronger response in the morphine-pretreated than in the methadone-pretreated rats, and sensitization of the response in some brain regions in the former. Moreover, the morphine pretreatment and the low-dose methadone pretreatment showed opposite effects on the Fos protein response in some layers of the cingulate and motor cortices. These results indicate that methadone has little propensity for inducing sensitization to opioids, whereas the reaction of an individual to methadone can be considerably enhanced by past opiate use.

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## Opposite effects of two endogenous tetrahydroisoquinolines: neuroprotective, 1-methyl-1,2,3,4-tetrahydroisoquinoline and neurotoxic 1-benzyl-1,2,3,4-tetrahydroisoquinoline on dopamine metabolism and its *in vivo* release in rat striatum

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1,2,3,4-Tetrahydroisoquinolines (TIQs) are present in mammalian brain and were regarded as endogenous toxins which may be responsible for the development of neurodegenerative diseases. 1-Benzyl-1,2,3,4-tetrahydroisoquinoline (1BnTIQ) was shown to possess neurotoxic properties, to cause parkinsonism-like syndrome in rodents and primates, and possibly to participate in pathogenesis of Parkinson's disease [Kotake et al., *J Neurochem*, 1996; Nagatsu and Yoshida *Neurosci Lett*, 1988]. The pharmacological properties of TIQs suggest that the compounds may have much greater potential than being merely substances for studying Parkinson's disease. Particularly interesting are their properties as antidopaminergic agents with atypical mechanism of action [Antkiewicz-Michaluk et al., *J Neural Transm*, 2000]. This suggests that TIQs may possess a potential as either atypical antipsychotics or agents useful in preventing drug use disorders. Among them, 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) is the most interesting and promising substance from clinical point of view. Our earlier

data have shown that 1MeTIQ has neuroprotective and antiaddictive properties [Antkiewicz-Michaluk et al., *J Neurochem*, 2006; Wąsik et al., *J Physiol Pharmacol*, 2007]. In this study, we compared the effects of a neurotoxic (1BnTIQ) and neuroprotective compound, 1MeTIQ on locomotor activity, dopamine metabolism and dopamine release *in vivo* in the rat using HPLC system for estimation dopamine (DA) and its metabolites.

Behavioral studies have shown that 1BnTIQ (50 mg/kg *ip*) induced a significant decrease in an exploratory locomotor activity but did not change the basal locomotor activity of rats. In contrast, 1MeTIQ (50 mg/kg *ip*) did not change the exploratory activity but significantly elevated basal activity of rats.

Biochemical study demonstrated that 1BnTIQ after a single injection produced an increase in DA metabolism through MAO-dependent oxidation pathway (a strong increase in DOPAC, HVA). At the same time, the level of 3-methoxytyramine (3-MT), an extraneuronal DA metabolite, was significantly de-

pressed, suggesting pathological, intraneuronal release of DA after 1BnTIQ administration. Interestingly, the effects of chronic administration of 1BnTIQ (10-day administration, 50 mg/kg *ip*) were much weaker indicating the development of tolerance to its DA-releasing effect. In contrast, 1MeTIQ administration did not change the rate of DA metabolism. At the same time, the DA oxidation rate was significantly decreased but the DA O-methylation rate was significantly increased by both acute and chronic administration of 1MeTIQ (50 mg/kg *ip*).

The *in vivo* microdialysis study in the rat striatum has demonstrated that 1BnTIQ after *ip* administration at a dose of 50 mg/kg significantly decreased DA re-

lease to an extracellular area but at the same time the concentration of its metabolites: DOPAC and HVA was strongly elevated. 1MeTIQ (50 mg/kg *ip*) slightly increased DA release but strongly elevated the concentration of its extraneuronal metabolite, 3-MT. Concomitantly, the concentration of DOPAC and HVA was decreased.

The present *ex vivo* and *in vivo* studies demonstrated that neuroprotective effects of 1MeTIQ may be explained by its capability to inhibit MAO-dependent oxidative catabolic pathway, and on the other hand, neurotoxic effect of 1BnTIQ may be connected with intraneuronal pathological release of DA.

## The effects of midazolam and buspirone on c-Fos protein expression in the brain of animals exposed to the open field test

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The aim of the study was to assess the effect of midazolam (0.5 mg/kg *ip*) or buspirone (1.5 mg/kg *ip*) on neuronal activity in the frontal (M2) and piriform cortex of rats exposed to open field test (neophobia-related behavior). One-way ANOVA revealed significant differences between groups in c-Fos expression in the M2 cortex [ $F(3, 27) = 12.08$ ;  $p < 0.01$ ], and the piriform cortex [ $F(3, 25) = 3.34$ ;  $p < 0.05$ ]. Newman-Keuls *post-hoc* test showed a significant increase in c-Fos expression in M2 area in the open field-exposed group (OFT-saline) compared to control group (saline) ( $p < 0.01$ ). This effect was attenuated by the administration of midazolam and buspirone 30 min before exposure of animals to the open field ( $p < 0.01$ ). In the piriform cortex, Newman-Keuls test showed in-

creased immunoreactivity in the control group exposed to the open field test (OFT-saline), compared to saline control group – the influence of neophobia alone. Additionally, the behavior of animals was analyzed. It was found that midazolam but not buspirone had no anxiolytic-like effect in the open field. Midazolam increased central distance crossed ( $p < 0.05$ ), and the number of central visits ( $p < 0.05$ ) in the open field test, compared to the control group.

These results indicate an important role of the frontal cortex M2 and piriform cortex in the neophobia-like reaction. Moreover, the frontal cortex may contribute to the antianxiety-like action of midazolam in the open field test.

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# Influence of sex differences and reproductive experience on rat behavior in the Morris water maze and in the elevated plus-maze test

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Hormones markedly affect the brain neuronal structure and function [Korol, *Neurobiol Learn Mem*, 2004]. The sex differences are important for spatial learning and memory processes, and modifying the emotional response in anxiety state [Blokland et al., *Behav Brain Res*, 2006; Jonasson, *Neurosci Behav Rev*, 2005].

Pregnancy and motherhood affect the hippocampal neurons and regulate the neuroprotection [Gatewood et al., *Brain Res Bull*, 2005; Kinsley et al., *Horm Behav*, 2006] The current work examined acquisition and reference memory in the Morris water maze test and evaluated anxiety in the elevated plus-maze test. We used the following groups of rats: I – male rats, II – NULL females (nulliparous – zero pregnancy and lactations), III – MULT females (3-multiparous pregnancies and lactations).

Our data showed that the percentage of time spent in open arms and percentage of entries to the open arm were comparable in male and female MULT rats in the elevated plus-maze test. We observed the higher anxiolytic activity in both above groups as compared

with the NULL females. The current work examined also acquisition of spatial learning and memory (i.e. escape latency time to reach the platforms, the distance traveled to reach the platform and swim speed over the 2 days of training).

We showed that males and MULT females acquired the spatial task significantly better than the NULL females. ANOVA with repeated measures showed that the performance (escape latency and distance) of MULT females and NULL females during the training sessions were better than in the first session. That effect was not observed in male rats. Moreover, we did not observe any changes in the time spent in the target quadrant in all investigated groups.

In conclusion, these data show important differences between males and females in cognitive effects. Pregnancy and mothering experience have a significant impact on spatial memory and anxiolytic activity.

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# Effects of serotonin (5-HT)<sub>2</sub> receptor ligands on the depressive-like effects associated with nicotine withdrawal in rats

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Nicotine dependence is a disease that impacts millions of individuals in the world. The main obstacle to the treatment of nicotine dependence is a strong withdrawal syndrome after its chronic use and relapses occurring even after long periods of abstinence [Schnoll

and Lerman, *Expert Opin Emerg Drugs*, 2006]. The most common withdrawal symptoms are: craving, depression, irritability, anxiety, difficulty in concentrating and increased appetite [Hughes, *Psychol Addict Behav*, 2007]. Recent studies demonstrate that sero-

tonin (5-HT) system and its receptors, mainly 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors (5-HT<sub>2A</sub>R and 5-HT<sub>2C</sub>R) may be crucial for modulating some behavioral effects of nicotine [Seth et al., Pharmacol Biochem Behav, 2002].

The present study sought to establish whether tonic or pharmacological activation of 5-HT<sub>2A</sub>R or 5-HT<sub>2C</sub>R could affect the animals' behavior in the forced swim test (FST, served as a preclinical model of depression) in either naive rats or those withdrawn from repeated nicotine treatment. We used a selective 5-HT<sub>2A</sub>R antagonist (M100,907; 60 min, *ip*), functional 5-HT<sub>2A</sub>R agonist (DOI; 60 min, *sc*), 5-HT<sub>2C</sub>R antagonist (SB242,084; 60 min, *ip*) and two selective 5-HT<sub>2C</sub>R agonists (Ro60-0175; 60 min, *sc* and WAY163,909; 60 min, *ip*). The above drugs were given acutely.

Administration of M100,907 (1–2 mg/kg), but not DOI (0.1–1 mg/kg) to naive rats decreased immobility time and enhanced swimming behavior without affecting climbing; these effects were not related to the changes in the basal locomotor activity. SB242,084 (0.3–1 mg/kg) reduced immobility time, without affecting swimming or climbing behaviors; its inhibitory effect paralleled the enhancement in animals' basal locomotion. Following Ro60-0175 (10 mg/kg) or WAY163,909 (1.5 and 10 mg/kg), a significant dose-dependent decrease in immobility and climbing and increase in swimming were observed. Both Ro60-0175 (3–10 mg/kg) and WAY163,909 (10 mg/kg) attenuated the basal locomotor activity.

In rats treated repeatedly (5 days) with nicotine (0.4 mg/kg/day *sc*) and then withdrawn, we found a significant increase in immobility time on the 1st, 3rd and 10th, but not on the 30th day of withdrawal. The maximum "pro-depressive" effect was observed on the 3rd day of withdrawal. For all the days tested, the basal locomotor activity of the animals was unaltered. On the 3rd day of nicotine withdrawal, treatment with M100,907 (1 mg/kg) produced a decrease in immobility time with no alterations in swimming or climbing behaviors. SB242,084 (0.3 mg/kg) induced a decrease in immobility time of the animals, prolonged swimming without affecting climbing behavior. Furthermore, Ro60-0175 (3 mg/kg) and WAY163,909 (0.75 and 1.5 mg/kg) decreased immobility time without effects on other tested behaviors.

Our data demonstrate that 5-HT<sub>2R</sub> ligands, mainly 5-HT<sub>2A</sub>R antagonist and 5-HT<sub>2C</sub>R agonists, exhibit effects that are typical of antidepressant drugs. Furthermore, 5-HT<sub>2C</sub>R agonists seem to abolish the effects of withdrawal from repeated nicotine treatment in rats. Our findings suggest that these compounds might be a useful treatment of nicotine addicts owing to their ability to suppress the withdrawal symptoms.

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