Posters

Effects of fatty acid amide hydrolase inhibitors on cocaine or food self-administration in rats

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Despite clear knowledge of the mechanism of action of cocaine, no effective therapy for cocaine addiction has been found [Carrera et al., Bioorg Med Chem, 2004]. Some empirical evidence suggests that ligands of the endocannabinoid (eCB) system may play an important role in cocaine addiction [Arnold, Pharmacol Biochem Behav, 2005]. The eCB, anandamide, acts as a retrograde messenger and is degraded by the enzyme fatty acid amide hydrolase (FAAH). Recent preclinical studies have indicated that the eCB system is involved in drug addiction. Thus, CB_1 receptors play a role in cocaine- or cue-induced cocaine seeking behavior [Xi et al., J Neurosci, 2006], while agents activating either directly or indirectly eCB system have been suggested to be a potential anti-relapse treatment of drug addiction [De Vries et al., Psychopharmacology, 2003].

The present study aimed to examine the effect of FAAH inhibitors, PMSF (15–120 mg/kg) or URB597 (0.1–3 mg/kg) and the selective CB_1 receptor antagonist AM251 (1–10 mg/kg) on the cocaine- or food-maintained self-administration and in the cocaine- or cue- or food-induced reinstatement in rats. Moreover, the spontaneous locomotor activity was studied following the above drug treatments.

Male Wistar rats were trained to self-administer cocaine (0.5 mg/kg/infusion) or food (sweetened milk) under a fixed ratio 5 schedule of reinforcement in 2-h daily sessions (6 days/week), then withdrawn from cocaine or food and tested for response reinstatement induced by cocaine (10 mg/kg, *ip*) or by the cue (light + tone), or by the food (sweetened milk). Spontaneous locomotor activity (expressed as a distance traveled in cm) was recorded for 2-h period using Optovarimex monitors.

PMSF (30–120 mg/kg), URB597 (0.1–3 mg/kg) or AM251 (1–10 mg/kg) did not alter cocaine self-administration. URB597 (3 mg/kg) or AM251 (1 mg/kg) significantly reduced the cocaine- or the cue-induced cocaine seeking behavior. The cue-induced reinstatement was inhibited by PMSF (120 mg/kg). PMSF administered at a dose of 60 mg/kg did not affect cocaine priming while its higher dose (120 mg/kg) combined with cocaine produced toxic effect. In food self-administration, PMSF (60 mg/kg) significantly reduced active lever presses, whereas neither URB597 (0.3–3 mg/kg) nor AM251 (3–10 mg/kg) altered it. Administration of AM251 (10 mg/kg) before PMSF (60 mg/kg) fully blocked the effects of PMSF in food self-administration. PMSF (120 mg/kg) or URB597 (3 mg/kg) significantly reduced, while AM251 (3–10 mg/kg) did not affect food seeking behavior. The inhibitory effect of URB597 (3 mg/kg) was blocked by AM251 (10 mg/kg), while the inhibitory effect of PMSF (120 mg/kg) was not altered by the administration of AM251 (10–30 mg/kg). PMSF, URB597 and AM251 did not produce alterations in spontaneous locomotor activity of rats.

Our results indicate that the inhibitory effects of FAAH inhibitors on cocaine seeking behavior are not specific and probably are related to a decrease in motivation for appetitive stimuli or other aspect of cocaine addiction.

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Effect of neonatal N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) treatment on antinociceptive effect produced by morphine, acetaminophen and nefopam in adult rats

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The sensation of pain in mammalians is known to be modified by endogenous pain inhibitory systems, predominantly through the descending noradrenaline (NA) and serotonin (5-HT) routes, and endogenous opioids such as β-endorphin and dynorphin. The activation of the descending pathways distinctly influences not only the release of glutamate from the primary afferent or interneurons, but also affects the release of GABA and glycine. Furthermore, the output of nociceptive information to the higher pain centers from projection neurons is also markedly altered. As a result, the severity of pain perception is drastically reduced. To study the influence of the central noradrenergic system on analgesia produced by morphine, acetaminophen and nefopam, intact rats were contrasted with rats in which noradrenergic nerves were largely destroyed shortly after birth with the neurotoxin DSP-4 [N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine; 50 mg/kg *sc* x2, P1 and P3]. At 10 weeks, tail flick, writing and Randall-Selitto tests were used to study the analgesic effects of morphine (5 mg/kg *sc*), acetaminophen (100 mg/kg *ip*) and nefopam (20 mg/kg *ip*). Additionally, 5-HT and dopamine (DA) synthesis rate in the thalamus with hypothalamus was estimated by an HPLC/ED method. The results of the present study showed that neonatal DSP-4 treatment markedly modified analgesia produced by the examined drugs. Also 5-HT synthesis rate after nefopam and acetaminophen administration was significantly affected by the chemical noradrenergic lesion.

A new source of dopamine in the brain

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Recent studies suggest that the brain cytochrome P450 (CYP) may play an important role in the metabolism of monoaminergic neurotransmitters (dopamine, serotonin), neurosteroids and arachidonic acid, as well as in the local biotransformation of drugs.

The aim of the present study was to identify and characterize rat CYP2D isoforms engaged in the hydroxylation of tyramine to dopamine, as well as to compare those isoforms with human CYP2D6 (HPLC with electrochemical detection). We also investigated the influence of selected antidepressant drugs (imipramine, fluoxetine, mirtazapine) on the activity of CYP2D, assessed by measuring the rate of bufuralol 1'-hydroxylation (HPLC with fluorescent detection). The study was conducted on rat brain microsomes and two biotechnologically different preparations of c-DNA-expressed CYPs: a) rat CYP2D1 and CYP2D2 and human CYP2D6, (Supersomes, Gentest; a high expression of NADPH P450 reductase); b) rat CYP2D4 and CYP2D18, (Bactosomes, Cypex; a low expression of NADPH P450 reductase).

Of the CYP2Ds isoforms tested, only CYP2D1 displayed no dopamine-forming activity. The efficacy of all the CYP2D isoforms engaged in dopamine formation was higher for m-tyramine than for p-tyramine. The affinity of tyramine for the CYP2D isoforms

tested (K_m) was as follows: CYP2D6 > CYP2D2 > CYP2D18 > CYP2D4 for m-tyramine, and CYP2D18 > CYP2D4 > CYP2D2 > CYP2D6 for p-tyramine. The V_{max} values were compared between isoforms with a similar expression of NADPH P450 reductase, i.e. between Supersomes (CYP2D6 > CYP2D2) and Bactosomes (CYP2D4 > CYP2D18), for both m- and p-tyramine. Brain microsomes catalyzed the hydroxylation of tyramine to dopamine; m-tyramine was more efficiently metabolized than p-tyramine. The reaction was inhibited by the CYP2D inhibitor quinine and anti-CYP2D4 antibodies, which indicates the contribution of CYP2D to dopamine formation in the brain. Fluoxetine and imipramine (but not mirtazapine) decreased the activity of CYP2D in brain microsomes. Using recombinant CYPs, we showed that fluoxetine and imipramine decreased the activity of CYP2D2 (K_i = 1.25 and 2.75 μM) more potently than that of CYP2D4 (K_i = 10 and 25 μM).

Our study provides a direct evidence that dopamine can be formed from tyramine by CYP2D isoforms in the rat brain. That alternative pathway may be inhibited by fluoxetine and imipramine, but not by mirtazapine which may affect the pharmacological profile of the antidepressant.

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Comparison of effects of antipsychotic drugs on dopamine D_1 and D_2 receptor interaction in the HEK 293 cells

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Dopamine D_1 and D_2 receptors have been long suggested to play a role in the pathophysiology and treatment of schizophrenia and these receptors are among several central neurotransmitter receptors for which antipsychotics have been shown to display a moderate affinity. Since endogenous dopamine has been suggested to influence the radioligand binding parameters [Seeman et al., Synapse, 1989], we report here the data concerning the affinity of risperidone and chlorpromazine for human dopamine D_1 and D_2 receptors in the model system, devoid of endogenous dopamine.

We examined the affinity of risperidone and chlorpromazine for dopamine D_1, D_2, and $D_2^{Ser311Cys}$ receptors, expressed alone or co-expressed concomitantly in HEK 293 cells. Binding assays were performed with [^{3}H]SCH23390 (D_1 receptor antagonist) and [^{3}H]spiperone (D_2 receptor antagonist). In competition analysis, risperidone and chlorpromazine were added at 10 concentrations ranging between 10^{-12} to 10^{-3} M.

The obtained results strongly suggest that there are two risperidone binding sites at the D_2 and $D_2^{Ser311Cys}$ receptors but there is only one binding site for D_1 receptor. However, if cells were transfected concomitantly with both D_1 and D_2 receptors, two binding sites were observed only if wild-type D_2 was present. D_1 receptor also displayed only one binding site for chlorpromazine, but – contrary to risperidone – when the cells were co-transfected with both dopamine receptors, two binding sites for chlorpromazine were observed.

These results might indicate the reciprocal allosteric influence of the receptors due to their direct interaction. To confirm that suggestion, we applied an advanced biophysical approach, namely, the dopamine receptors were tagged with fluorescence proteins (that modification did not change the binding pa-

rameters), their interaction was studied by fluorescence resonance energy transfer (FRET) detected by fluorescence lifetime measurements in the living cells [Dziedzicka-Wasylewska et al., Biochemistry, 2006]. The results obtained in the present study indicate that both risperidone and chloropromazine, like clozapine, promoted the uncoupling of D_1-D_2 heterodimers, but, in contrast to clozapine, that effect was significant at higher doses of the drugs.

Neonatal N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) treatment impairs serotonin $(5\text{-HT})_{1B}$ receptor reactivity in adult rats. Biochemical studies

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Several studies have shown the existence of a mutual interaction between norepinephrine and serotonin in the brain and periphery. Formerly, we found that chemical lesioning of noradrenergic neurons with N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) greatly affected central dopaminergic system functioning (e.g. reactivity of dopamine D_1 and D_2 receptors). We also demonstrated that the neonatally DSP-4-treated rats developed desensitization of serotonin $(5\text{-HT})_{1A}$ autoreceptors in adulthood. At present, there are no data concerning the influence of DSP-4 treatment on reactivity of the 5-HT_{1B} receptors. For this reason, noradrenaline (NA), 5-hydroxytryptamine (5-HT), 5-hydroxyindole-3-acetic acid (5-HIAA), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) contents were examined in the prefrontal cortex, striatum and hypothalamus after administration of 5-HT_{1B} ligands (agonist; CP 94253 4 mg/kg *ip* and antagonist SB 216641 4 mg/kg *ip*). Furthermore, 5-HT synthesis rate was examined in the prefrontal cortex, striatum, hypothalamus, and a microdialysis study (medial prefrontal cortex) was performed.

We demonstrated that 5-HT synthesis rate was significantly attenuated in the prefrontal cortex and striatum after CP 94253 administration, and SB 216641 was able to antagonize this effect in the tested brain areas of control animals. No changes in 5-HT synthesis rate were observed in the hypothalamus. CP 94253 failed to significantly inhibit 5-HT synthesis rate in all examined brain structures of DSP-4-treated rats. In the microdialysis study, CP 94253 induced a long-lasting attenuation of 5-HT release in the medial prefrontal cortex of control rats, being without effect in DSP-4 lesioned animals.

The role of the accumbal dopamine D_1, D_2 and D_3 receptors in the spontaneous alternation behavior in rats

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The spontaneous alternation (SA) model is based on a natural tendency of rats to explore two arms of the T-maze sequentially and in succession. Thus, the paradigm consists of a choice situation which, if disturbed under specific pharmacological conditions, leads to perseverant responses, and therefore, it is believed to model some aspects of human obsessive-compulsive disorder (OCD), namely, indecisiveness and perseverance. OCD is a complex psychiatric disorder with a lifetime prevalence of up to 3% of the general population. Despite a great number of preclinical and clinical studies, which have been conducted during the recent decades, the pathophysiology of OCD and its treatment is still far from being clear and satisfactory. Based mainly on the clinical efficacy of serotonin reuptake inhibitors (SRIs), it has been believed that OCD may be related to the functions of the brain serotonin system. However, recently a growing number of evidence suggest that mesolimbic dopamine system may also be involved in the mechanisms of OCD. Therefore, the present study was designed to evaluate the effect of agonists of various dopamine receptors, microinjected into the shell or core of the nucleus accumbens, in the SA model.

Quinpirole and PD128,907 (both at 0.1–5 μg/site, 15 min before the session), injected into the core and shell of the nucleus accumbens, produced a dose-dependent impairment of the SA behavior. Similar reduction in the SA responses was caused by SKF81297 when it was injected into the shell but not the core of the nucleus accumbens. The impairment of the SA behavior produced by intra-accumbens quinpirole, PD128,907 and SKF81297 was effectively attenuated by a subacute (3 times) pretreatment with clomipramine and escitalopram, but not with imipramine (all at 10 mg/kg, *ip*).

These results are consistent with recent developments in the clinical pharmacotherapy of OCD and provide further support for the usefulness of SA procedure as an experimental tool for investigating the physiological and neurochemical mechanisms underlying OCD and its treatment. They also indicate the involvement of accumbal dopamine D_1, D_2 and D_3 receptors in the control of the SA behavior, which is in agreement with other evidence suggesting a dysfunction of mesolimbic dopaminergic neurotransmission in OCD.

Nitric oxide is not involved in the vasopressin effects in social recognition in rats

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The estimation of the relationship between neuropeptides and neuronal nitric oxide synthase (NOS) as well as its specificity in different types and stages of memory has been studied by us for several years. The potential role of neuronal nitric oxide synthase in vasopressin effects on social memory has aroused a great deal of our interest and was the subject of our current study. The social memory is a form of non-spatial memory which has an essential meaning in the cognitive function. The nature and neurobiological bases of

social memory have been investigated for many years [Adolphs, Cur Opin Neurobiol, 2001; Lieberman, Neuroimage, 2005]. This view is confirmed by results obtained from clinical human studies [Kirsch, Epilepsy Behav, 2006; Penn et al., Schizophr Res, 2005; Williams et al., Arch Clin Neuropsychol, 2005].

Therefore, we decided to test the hypothesis that NO participates in the facilitating effect of arginine vasopressin (AVP) on social recognition. We evaluated the behavioral effects of AVP at a dose of 0.1 μg after the inhibition of neuronal nitric oxide synthase by an intraperitoneal injection (*ip*) of 7-nitroindazole (7-NI) at the dose of 30 mg/kg in the social discrimination test [Penn et al., Schizophr Res, 2005; Popik and Van Ree, Prog Brain Res, 1998; Thor and Holloway, J Comp Physiol Psychol, 1982]. Inhibition of the nNOS was evoked immediately after the first exposure to a juvenile rat (learning trial), 120 min before the retrieval trial. Fifty minutes after NOS inhibition, the animals were given *icv* a solution containing vasopressin. AVP had a tendency to improve the effect on the social discrimination but the influence was not significant as compared to the control group. On the other hand, vasopressin in a significant manner improved social discrimination in comparison with 7-NI(alone)-pretreated rats. The administration of 7-NI did not change the behavioral effects of AVP in this paradigm.

Our results suggest that the central action of AVP on social discrimination is independent of NO synthesis in the brain.

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Subchronic exposure to the pesticide rotenone potentiates the effect of intranigral administration of the proteasome inhibitor lactacystin on striatal dopamine metabolism in rats

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A growing body of evidence suggests that Parkinson's disease (PD) may be caused by numerous factors, both environmental and genetic. Epidemiological research has shown a correlation between the exposure to pesticides and PD. On the other hand, genetic and biochemical studies have demonstrated a critical role of ubiquitin-proteasome system impairment and subsequent proteolytic stress in the pathogenesis of PD. Based on the multifactorial etiology of PD, the present study was aimed at ascertaining how combined administration of the mitochondrial complex I inhibitor rotenone and the proteasome inhibitor lactacystin affects the striatal dopamine (DA) level and its catabolism in rats.

Four groups of male Wistar rats were used for the present study. The animals were given rotenone (1 mg/kg *sc*) systemically for 15 days, and lactacystin (1 or 5 μg/2 μl) unilaterally into the substantia nigra pars compacta, alone or in combination with rotenone. On day 15 of rotenone treatment (7 days after lactacystin), the animals were killed by decapitation and their striata were dissected on an ice-chilled plate. The levels of DA and its metabolites were assayed in striatal homogenates using an HPLC method with electrochemical detection.

No differences were found in the levels of striatal DA in rats treated with rotenone alone; on the other hand, significant increases were observed in 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels, as well as in the DOPAC/DA and HVA/DA ratios, but there was a decrease in 3-MT level and the 3-MT/DA ratio. Lactacystin alone (1 or 5 μg/2 μl) produced either a moderate (56%) or a dramatic (85%) drop in DA content. Similarly, its higher dose caused larger decreases in the levels of DA metabolites. As regards DA catabolism, either dose of

lactacystin evoked a potent acceleration of monoamine oxidase (MAO)-dependent oxidative DA deamination, catechol-O-methyltransferase (COMT)-dependent O-methylation and total DA catabolism in the left striatum. After combined treatment, a tendency towards further decrease in DA content was observed in the left striatum compared to the lactacystin-treated groups. There was also a significant decrease in the concentration of the extraneuronal DA metabolite 3-MT, but no further declines were seen in DOPAC and HVA levels. After combined treatment, the metabolic ratios of DOPAC/DA and HVA/DA were markedly higher than those reported for groups receiving lactacystin or rotenone only.

The obtained results show that despite the fact that both lactacystin and rotenone affect very significantly DA catabolism, only lactacystin administration leads to a diminution in striatal DA content. Combined administration of the two compounds potentiates DA catabolism but has a less profound effect on striatal DA level.

The influence of the acute and chronic administration of a pesticide paraquat on the reactive oxygen species in rats

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Parkinson's disease (PD) is a slowly progressing neurodegenerative disease caused by the loss of dopaminergic cells in substanta nigra (SN) and decreased levels of dopamine (DA) in the caudate nucleus and putamen (CP). It presents with slowness of movement, akinesia, tremor and postural disturbance. Actual causes of this degeneration are still unknown but there have been described positive correlations between the incidence of PD and use of pesticides, e.g. paraquat (PQ). Our recent study [Ossowska et al., Eur J Neurosci, 2005] has shown that a long-term PQ administration can be used as an animal model of progressing PD.

In the present study, we examined the effects of both acute and chronic PQ administration (10 mg/kg *ip* once a week for 4 weeks) on the production of reactive oxygen species (ROS) in brain tissue (CP, SN, prefrontal cortex – PFC) and in peripheral organs (liver, kidneys, lungs, muscles). The measurements were done by HPLC analysis of salicylic acid metabolites 2.3DHBA and 2.5DHBA, markers of hydroxyl radical formation.

We observed that there were no major changes after a single administration of PQ, only a tendency to increased 2.3DHBA-to-salicylic acid ratio in muscles ($p = 0.093$) and liver ($p = 0.089$). After 4 injections of PQ, the 2.3DHBA-to-salicylic acid ratio increased in CP and showed increasing tendencies in SN and FC. More interestingly, the levels of salicylic acid in the brain were decreased after chronic PQ treatment suggesting changes in blood-brain barrier (BBB) or PQ-induced changes in its metabolism. In peripheral organs, only in the lungs the salicylic acid levels dropped significantly and 2.3DHBA-to-salicylic acid ratio was decreased both in the lungs and liver. The 2.5DHBA marker is also a metabolite of salicylic acid but it can be formed both by reaction with hydroxyl radical as well as by enzymatic reaction catalyzed by cytochrome P450. Its levels in muscles were decreased after a single PQ administration. After chronic PQ administration, 2.5DHBA was also decreased in the muscles, lungs and kidneys and in CP and PFC.

The above results seem to suggest that PQ administered chronically at a low dose increases ROS production and induces possible changes in BBB. The differences between the brain and in peripheral tissues are discussed.

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Effects of intracerebral administration of the selective proteasome inhibitor lactacystin on dopamine metabolism in the striatum and on the number of tyrosine hydroxylase immunoreactive neurons in the substantia nigra pars compacta

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Progressive loss of nigrostriatal dopaminergic neurons, accompanied by the formation of intracytoplasmic proteinaceous inclusions known as Lewy bodies, is one of the cardinal pathological features of Parkinson's disease (PD). Although specific causes for the degeneration of dopaminergic neurons still remain unclear, a vast body of evidence have implicated oxidative stress, mitochondrial dysfunction, protein mishandling and inflammation as possible pathological factors. Impairment of the ubiquitin proteasome system (UPS), which is responsible for the degradation and elimination of damaged, oxidized, nitrated and misfolded proteins, has recently been proposed as a potential etiopathogenic factor for PD. However, the application of proteasome inhibitors as model substances producing a parkinsonian-like syndrome in animals seems controversial, since some authors have shown that systemic administration of these compounds to rats evokes symptoms characteristic of the disease, while other researchers have not been able to reproduce these effects. In neurons, the majority of proteasome activity is related to cell bodies, and only its small percentage is localized to their neuritic processes.

The aim of the present study was to determine whether the selective proteasome inhibitor lactacystin differently affects the striatal level of dopamine (DA) and its catabolism after administration of that compound into the striatum, i.e. in the vicinity of DA terminals, or into the substantia nigra pars compacta (SNc), i.e. directly into dopaminergic cell bodies. Moreover, it was also checked whether such treatment evokes changes in the number of tyrosine hydroxylase immunoreactive neurons (TH-ir) in the SNc. Experiments were performed on male Wistar rats which were injected unilaterally with lactacystin (0.5–10 μg/2 μl) into the left striatum or SNc under pentobarbital anesthesia. Control rats received a solvent instead of lactacystin. One to three weeks after surgery, the animals were killed by decapitation and their striata were dissected on an ice-chilled plate. The levels of dopamine (DA) and its metabolites were assayed in striatal homogenates using an HPLC method with electrochemical detection. TH-ir neurons were counted stereologically in the SNc.

There were no significant differences in the concentrations of DA and its metabolites between the left and the right striatum one, two and three weeks after unilateral intrastriatal lactacystin (10 and 5 μg/2 μl) injection. In contrast, one week after unilateral intranigral administration of lactacystin at high doses (5 and 2.5 μg/2 μl), a dramatic decrease in the levels of DA (by 85% and 77%, respectively) and its metabolites was observed in the left striatum. After lower doses (1 and 0.5 μg/2 μl), the decrease in DA level on the lesioned side was, respectively, either moderate (by 56%) or small (by 25%). As regards DA catabolism, intranigral injection of lactacystin at all the examined doses except for a dose of 0.5 μg/2 μl evoked acceleration of MAO-dependent oxidative DA deamination and enhancement of COMT-dependent O-methylation in the left striatum. As shown by a stereological study, lactacystin at doses of 5 and 2.5 μg/2 μl, administered into SNc, also produced a marked loss of TH-ir neurons in that structure. The obtained results show that only intranigral, but not intrastriatal, administration of lactacystin evokes distinct changes in the striatal level of DA and its metabolites. The latter effects may stem from the degeneration of dopaminergic neurons in the SNc. These results also show that DA cell bodies in the SNc, but not DA terminals in the striatum, are susceptible to the impact of proteasome inhibition.

The role of nitric oxide in the mechanism of porphyrin-induced seizures

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It was previously demonstrated that synthetic *meso*-tetra-4N-methylpirydyl-porphyrin (P) exerted a convulsive effect in rats and mice. This effect was blocked by propranolol, a β-adrenergic antagonist and by high doses of diazepam, an agonist of benzodiazepine receptor. In this report, we present the results of our study on the role of nitric oxide in the mechanism of P-induced convulsions. The experiments were performed on two species: male Balby mice and female Wistar rats. The following measures were used to determine a convulsive effect: the percentage of animals with seizures, the number of seizure episodes/2 h, the latency time of the beginning P-induced seizure activity and P-induced seizure activity determined by Racine's score. Convulsive effect was observed in mice after *ip* administration of P at the dose of 50 μmol/kg (59.3 mg/kg) and 100 μmol/kg (118.7 mg/kg) and in rats after its administration into the lateral brain ventricle (*icv*) at the dose of 14.2 nmol. Pretreatment of mice with L-NAME (10 and 40 mg/kg *ip*) did not inhibit convulsive effect, but increased this effect of P.

On the other hand, pretreatment of mice with methylene blue (5 mg/kg *ip*) inhibited the effect of the lower dose of P (50 μmol/kg) but not of the higher dose (100 μmol/kg *ip*). Methylene blue also slightly modified convulsive effect of P applied *icv*. The obtained results suggest a low significance of $NO^{\bullet}$ in the mechanism of convulsive effect of P.

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Oxidative status of the cerebrum and cerebellum of rats exposed to lead or cadmium

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Lead (Pb) and cadmium (Cd) are heavy metals eliciting neurotoxic action. In spite of numerous neurotoxicological studies, the mechanisms of their impact on the central nervous system (CNS) are not completely explained. The CNS is especially susceptible to oxidative damage. Brain consumes large amounts of oxygen and contains cellular membranes rich in long-chain polyunsaturated fatty acids. Moreover, it has a weak enzymatic and non-enzymatic antioxidant barrier.

Thus, the aim of the present study was to estimate the influence of exposure to Pb or Cd on the chosen parameters of oxidative status in the cerebrum and cerebellum. Wistar rats were administered 500 mg Pb/dm^3 (as lead acetate) or 50 mg Cd/dm^3 (as cadmium chloride) in drinking water for 12 weeks. Control rats drank redistilled water throughout the experiment. At termination, whole brain without medulla oblungata was dissected. The cerebrum and cerebellum were separated. The concentration of thiobarbituric acid-reactive substances (TBARS), the activities of superoxide dismutase (SOD) and catalase (CAT) as well as the concentrations of reduced glutathione and vitamin E (α-tocoferol) were determined in the cerebrum and cerebellum homogenates.

The exposure to Pb or Cd led to an increase in the activities of SOD and CAT in the cerebrum with a concomitant decrease in the concentrations of GSH and vitamin E but had no influence on the TBARS concentration.

In the cerebellum of rats exposed to Pb or Cd, an increase in the TBARS concentration and a decrease in the activity of SOD were observed. Moreover, in the Pb-exposed rats, a decrease in the activity of CAT was noted, as well. Both Pb and Cd decreased the cerebellar concentrations of glutathione peroxidase (GSH) and vitamin E.

The results show that exposure to Pb or Cd leads to the changes in the activity of antioxidative enzymes and in the concentration of non-enzymatic antioxidants in the cerebrum and cerebellum. The cerebellum seems to be more susceptible to the oxidative impact of Pb and Cd than the cerebrum. The results justify continuation of this study.

The role of the central noradrenergic system in the sensation of pain in rats

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Pain is a homeostatic mechanism that intervenes to protect the organism from harmful mechanical, chemical, and thermal stimuli that could damage its integrity. The role of opioids, either endogenous and exogenous, in pain pathophysiology has been thoroughly examined and confirmed. Conversely, there is also a large body of evidence demonstrating involvement of the central noradrenergic system in regulation of the sensation of pain, for instance, the activation of α_2-adrenoceptor was demonstrated to produce a profound analgesic action in numerous pain models. Furthermore, behavioral studies have shown that α_2-adrenoceptors mediate spinal analgesia and adrenergic-opioid synergy, which may be useful in clinical pain management. Certain α_2-receptor agonists are also clinically used in patients suffering from various types of pathological chronic pain, including neuropathic pain. Recently, we demonstrated that N-(-2--chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) injected two times on the 1st and 3rd days of postnatal life at a dose of 50 mg/kg *sc* a day resulted in a profound noradrenergic system lesion; endogenous noradrenaline (NA) content in the frontal cortex was reduced by approximately 95% and in the hippocampus by 98% without impairing dopaminergic and serotonergic neurons. Employing this model, we examined the analgesic effects of morphine (5 mg/kg *sc*), tramadol (20 mg/kg *ip*) and imipramine (10 mg/kg *ip*) in three animal models of pain (tail flick, Randall-Selitto and formalin tests). Moreover, serotonin (5-HT) and dopamine (DA) synthesis rate was estimated in the brainstem. We found that sensation of pain elicited by chemical, mechanical or thermal stimuli was markedly affected in DSP-4-treated rats. The analgesic effect (attenuation or intensification) depended on the examined drugs and the test employed in a specific study. Also 5-HT synthesis rate analyzed after morphine treatment in brainstem was modified in the DSP-4 group. In conclusion, these studies highlight the importance of NA in the modulation of pain in rats.

Histamine level in the brain of neonatally 6-OHDA-lesioned rats (rodent model of Parkinson's disease)

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Rats lesioned shortly after birth with 6-OHDA have been proposed to be a near-ideal model of severe Parkinson's disease, because of non-lethality of the procedure, near-total destruction of nigrostriatal dopaminergic fibres and near-total dopamine (DA) denervation of the striatum [Kostrzewa et al., J Neural Transm, 2006]. There are scarce data that in Parkinson's disease, histaminergic activity is increased in the brain [Rinne et al., J Neurochem, 2002]. Therefore, the aim of this study was to determine histamine (H) content in the brain of rats which model Parkinson's disease.

At 3 days after birth, Wistar rats were pretreated with desipramine (20 mg/kg *ip*) 1 h before bilateral *icv* administration of 6-OHDA (67 μg base, on each side) or saline-ascorbic acid (0.1%) vehicle (control). At 8 weeks the levels of DA and its metabolites (DOPAC, HVA) were determined in the striatum and frontal cortex by HPLC/ED technique. Also in the hypothalamus, hippocampus, frontal cortex and medulla oblongata the level of histamine was analyzed by an immunoenzymatic method.

We confirmed that 6-OHDA decreased dramatically the level of DA and its metabolites in the rat striatum and frontal cortex at 8 weeks. Also, the histamine level was increased significantly in the hypothalamus, hippocampus and medulla oblongata. These findings demonstrate that the histaminergic system is altered in the brain of rats, lesioned to model Parkinson's disease.

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Effect of histamine receptor antagonists on oral activity and stereotyped behaviors in neonatally 6-OHDA-lesioned rats (rodent model of Parkinson's disease)

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Rats lesioned shortly after birth with 6-OHDA have been proposed to be a near-ideal model of severe Parkinson's disease, because of non-lethality of the procedure, near-total destruction of nigrostriatal dopaminergic fibers and near-total dopamine (DA) denervation of the striatum [Kostrzewa et al., J Neural Transm, 2006]. There are scarce data that in Parkinson's disease activity of the central histaminergic system is increased [Rinne et al., J Neurochem, 2002]. Therefore, the aim of this study was to examine the effect of histamine receptor antagonists on dopamine receptor agonist-induced oral activity and stereotyped behavior in an animal model of Parkinson's disease.

At 3 days after birth, Wistar rats were pretreated with desipramine (20 mg/kg *ip*) 1 h before bilateral *icv* administration of the catecholaminergic neuro-

toxin 6-OHDA (67 μg base, on each side) or saline-ascorbic acid (0.1%) vehicle (control). At 8 weeks, levels of DA and its metabolites (DOPAC, HVA) were estimated in the striatum and frontal cortex by HPLC/ED technique.

In separate groups (6-OHDA-lesioned and control rats) oral activity was induced by the DA D_1 agonist SKF 38393 (1 mg/kg *ip*), while stereotyped behavior was induced by the nonselective DA agonist apomorphine (1 mg/kg *ip*). Numbers of oral movements were counted during 1 h observation after SKF 38393 [Brus et al., J Pharmacol Exp Ther, 1994], and stereotyped behavior was assessed by a scoring method [Creese and Iversen, Brain Res, 1973] during 90 min. S(+)chlorpheniramine (10 mg/kg *ip*), cimetidine (5 mg/mg *ip*) or thioperamide (5 mg/kg *ip*), respective H_1-, H_2-, or H_3 receptor antagonists, were administered 1 h before SKF 38393 or apomorphine. We found that all three histamine receptor antagonists diminished oral activity in 6-OHDA-lesioned rats *vs*. control, with thioperamide having the greatest effect. Only thioperamide prevented apomorphine-induced stereotyped behavior in 6-OHDA-lesioned rats. These findings indicate that histaminergic neurons exert a modulatory role in Parkinsonian 6-OHDA-lesioned rats.

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Effects of CRF and α-helical $CRF_{(9–41)}$ on rat fear responses, c-Fos and CRF expression in brain structures

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The aim of this study was to examine the influence of intracerebroventricullary administered CRF, and a non-selective CRF receptor antagonist, α-helical $CRF_{(9–41)}$, on freezing reaction in the conditioned fear test, expression of c-Fos and CRF in brain structures, and serum corticosterone concentration. It was found that pretreatment of rats with CRF (1 μg/rat), enhanced freezing response, and conditioned fear-elevated serum corticosterone concentration. Moreover, exogenous CRF increased aversive context-induced expression of c-Fos in the parvocellular neurons of the paraventricular hypothalamic nucleus (pPVN), CA1 area of the hippocampus, and M1 area of the frontal cortex. Immunocytochemical study showed also an increase in the expression of CRF in the pPVN, and central and medial nucleus of the amygdala, 35 min after corticoliberin administration and 10 min after the test. In pPVN, this effect was upheld for 115 min after drug injection. The opposite pattern of behavioral and biochemical changes was present after pretest α-helical $CRF_{(9–41)}$ administration (10 μg/rat), namely, a decrease in fear reaction and serum corticosterone concentration, attenuation of fear-induced c-Fos expression in the dentate gyrus, CA1 area of the hippocampus, Cg1, Cg2, and M1 area of the frontal cortex, decline in the CRF expression in the M2 cortical area, were enhanced 10 min after the test. In conclusion, the present data suggest that the M1 area of the frontal cortex, and CA1 area of the hippocampus may facilitate the function of HPA axis, thus finally leading to corticosterone release. The obtained results underline also the role of the frontal cortex (M2 area) in mediating the effects of CRF on the conditioned fear response.

Influence of drugs acting on nitricoxidergic system on tolerance to sedative effect of diazepam in mice

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Classical benzodiazepines are widely used as hypnotics, anxiolytics and anticonvulsants. They are known to enhance the γ-aminobutyric acid (GABA)ergic neurotransmission through binding to the specific, benzodiazepine recognition site within $GABA_A$ receptor-ion channel complex, and allosterically modulate its activity. There is a line of evidence indicating that a long-term administration of benzodiazepines results in the development of tolerance to some effects of these drugs (including their sedative, muscle relaxant, and anticonvulsant effects) even at low (therapeutic) doses, a phenomenon that limits their clinical efficacy. Such long-term treatment is also associated with the development of physical dependence. Although the molecular bases for these phenomena remain uncertain, tolerance and dependence appear related to the pharmacodynamics, rather than to pharmacokinetics of benzodiazepines.

Nitric oxide (NO) is enzymatically produced in response to activation of excitatory amino acid receptors. Glutamate released from presynaptic terminals acts upon N-methyl-D-aspartate receptors and promotes NO synthase activity and formation of NO from L-arginine. NO acts as an endogenous activator of guanyl cyclase thereby increasing the level of an intracellular second messenger, cyclic guanylate cyclase (cGMP). Recent studies indicate that NO may play a role in tolerance and dependence on substances, such as opioids, ethanol, psychostimulants and nicotine.

Literature data point to the relationship between L-arginine: NO: cGMP pathway and GABA-mediated transmission in the central nervous system. A number of *in vivo* and *in vitro* studies suggest that NO plays a modulatory role in either release or uptake of neurotransmitters including glutamate and GABA. It has also been postulated that NO can modulate the activity of $GABA_A$ receptors or act directly on $GABA_A$ receptors.

The present studies were undertaken to determine the role of NO in the development and expression of tolerance to the sedative effect of diazepam. Mice were treated chronically with diazepam (15 mg/kg/day) *sc* for 8 days. On 9th day they were injected with diazepam (10 mg/kg) and their level of motor activity was tested 30 min thereafter. The effect of a non-selective NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME- 50, 100 mg/kg *ip*), selective inhibitor of neuronal NO synthase: 7-nitroindazole (10, 20 mg/kg *ip*) and the substrate for NO formation (L-arginine- 125, 250 mg/kg *ip)* on the development and expression of tolerance to sedative action of diazepam was examined in mice. Locomotor activity was assessed on the 1st and 9th day of experiment. L-NAME, 7-nitroindazole and L-arginine, were administered 35 min before the test. The experiments were carried out on male albino Swiss mice and were performed in accordance with the ethical requirements. Data were evaluated using one-way ANOVA. A level of $p < 0.05$ was considered statistically significant.

In the present studies, we have shown that chronic administration of diazepam resulted in the development of tolerance to sedative action of diazepam. We have observed that administration of the NO synthase inhibitors, L-NAME and 7-nitroindazole, with diazepam for 8 days attenuated the development of diazepam tolerance. We have also observed an inhibiting effect of NO synthase inhibitors on the expression of tolerance to sedative effect of diazepam. Co-administration of L-arginine, an endogenous donor of NO, with diazepam had no effect on either development or expression of tolerance to diazepam in mice.

The above results suggest that the NO may be involved, at least partly, in the development and expression of tolerance to diazepam.

The changes in GABAergic neurotransmision system in mGlu7 receptor deficient mice

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Broad evidence indicates that interaction between the GABAergic and glutamatergic system could be an efficient way to achieve antidepressant and/or anxiolytic activity. The aim of our study was to investigate the changes in GABAergic system in mice lacking the mGlu7 receptor. The levels of GAD 65, GAD 67 and reeline protein were investigated using immunohistochemistry and Western blotting method. The levels of these proteins were measured in the hippocampus of wild-type, heterozygous and homozygous mice. The levels of mRNA were also investigated using *in situ* hybridization. We found that the levels of GAD 67 were decreased both in hetero- and homozygous mice and the level of reeline protein was increased. Using Western blotting method, we found that the level of GAD 65 was not changed. These preliminary results strongly suggest that there can be an interaction between these two neurotransmitter systems and that mGlu7 receptor is strongly engaged in these processes.