Poster presentations

SESSION: Pharmacology of the central nervous system

An endogenous neurotoxin, 1-benzyl-1,2,3,4-tetrahydroisoquinoline impairs of L-DOPA-induced behavioral and biochemical effects on dopamine system in rat

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1-Benzyl-1,2,3,4-tetrahydroisoquinoline (1BnTIQ), an endogenous neurotoxin was identified in mouse brain and cerebrospinal fluid (CSF) of normal human subjects, however in Parkinson's disease (PD) patients its concentration in CSF is fortified [Kotake et al., J Neurochem, 1995]. Additionally, it was demonstrated in experimental studies that peripheral administration of 1BnTIQ causes a parkinsonism-like syndrome in rodents and primates. Recently, our experiments have shown that a single injection of 1BnTIQ produced a significant decrease in an exploratory locomotor activity, and a dramatic fall dopamine concentration in the brain. Interestingly, multiple 1BnTIQ treatments (50 mg/kg/day, *ip* × 10 days) resulted in development of tolerance to its dopamine depressing effect while the impairment of dopamine synthesis was persisted [Wąsik et al., Neurotox Res, 2009]. It is well known that L-DOPA is the main medication used for the treatment of Parkinson's disease, and in this study we evaluated the effects single and multiple 1BnTIQ (25 and 50 mg/kg/day, *ip* × 14 days) administration on L-DOPA (100 mg/kg, *ip* + Carbidopa 10 mg/kg, *ip*)-induced changes in motor activity and dopamine metabolism in the extrapyramidal brain structures (substantia nigra and striatum) of rat. The experiments were carried out on male Wistar rats weighing 250–280 g. The biochemical data were established by HPLC methodology with electrochemical detection.

Results: the behavioral experiments have shown that both single and multiple administration of 1BnTIQ completely antagonized L-DOPA-induced an increase of horizontal and vertical locomotor activity in rats. Biochemical studies demonstrated that L-DOPA (100 mg/kg, *ip* + Carbidopa 10 mg/kg, *ip*) produced a strong increase of the rate of dopamine metabolism (by about 500%, $p < 0.001$), dopamine concentration (by about 200 to 300% of control level, $p < 0.001$), and a considerable much higher increase of the level its metabolites in striatum and substantia nigra. Both, acute and chronic administration of 1BnTIQ significantly antagonized all biochemical effects produced by L-DOPA injection.

Conclusion: the present studies demonstrate that the stimulatory effect of L-DOPA on dopamine system was strongly impaired by 1BnTIQ. In connection therewith fact that 1BnTIQ is an endogenous substance present in the brain its concentration in PD patients may be a very important factor for efficacy of L-DOPA therapy in clinic.

Effect of nefopam on nigrostriatal system in rats. Behavioral and biochemical studies

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It is well known that besides typical symptoms of Parkinson disease, the non-motor disabilities were found to be the major contributing factors to impairments in disease-related quality of life. The scope of non-motor manifestations of Parkinson disease is broad, and includes depression, pain, disturbances in mood, cognition, autonomic function, sleep, perceptual changes and impulse control. Pain as a primary symptom is usually located on the side of the body that is most compromised by the disease. The treatment always demands a great adjustment of dopamine agonist, local injections of steroids, massages, physiotherapy and analgesic therapy, which improve the life quality of patients. The aim of this study was to examine biological effects of nefopam – non-opioid analgesics drug in rats. The battery of behavioral tests were employed to assess the impact of neopam on dopaminergic system. Furthermore dopamine synthesis rate in the frontal cortex, nucleus accumbens and striatum after nefopam challenge as well as the microdialysis of the striatum was performed. It has been determined that nefopam administered in doses of 1.0; 5.0; 10; 20 and 40 mg/kg, *ip* (intraperitoneal) was without effect on locomotor activity in rats although higher doses (20 or 40 mg/kg, *ip*) evoked stereotypy behavior. Nefopam ameliorated motor coordination (assessed in rota-rod test) and diminished cataleptogenic effect of SCH 23390. In biochemical studies it has been shown that nefopam reduced dopaimne synthesis rate in the frontal cortex, nucleus accumbens and striatum, and augmented dopamine release in the striatum in rats. The data of the present study lead to the proposal that "behavioral-biochemical profile" of this analgesics justify its use in patient with motor abnormalities e.g. in Parkinson disease.

Effect of candesartan on the chronic stress-induced cognitive impairment in rats

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Negative effects of prolonged stress on memory are increasingly important. People undergoing chronic stress suffer from the variety of cognitive and mood disturbances including impairments of associative and spatial memory, concentration, attention and also anxiety. In rats, a stress-induced overexpression of the AT1 angiotensin receptors in brain has been found to participate in several negative effects of chronic stress such as gastric ulcers, pathologic cardiovascular functioning including hypertension, and the damage of certain brain structures including hippocampus. In this study we searched for the protective effects the AT1 angiotensin receptor blockade with candesartan against the adverse effects of chronic immobilization stress on memory in rats. Two groups of male Wistar rats (140–160 g, n = 14–16) were chronically stressed by keeping them daily (2 h/21 days) in tight plastic tubes. The subjects of the group 1 received candesartan (0.1 mg/kg, orally) each day before the stressing procedure. The rats of the group 2 received vehicle (0.5% methylocellulose solution). Another two groups of rats (3 and 4) receiving candesartan and vehicle, re-

spectively, were appropriately handled but not stressed. Next day after ending the chronic stress procedure all rats were tested in three cognitive paradigms: passive avoidance (PA), object recognition (OR) and the Barnes maze (BM). Stressed animals displayed decreased recall of the PA behavior ($p < 0.01$), decreased OR ($p < 0.05$) and an impairment of reference memory in the BM. These effects were not seen in the animals stressed but pre-treated with candesartan. Open field test applied to control for the unspecific motor effects of our treatments and procedures showed no influence of stress and candesartan on the animals' performance.

In conclusion, these data strongly suggest that the AT1 angiotensin receptor blockade effectively counteracts deleterious effects of stress on memory.

Cholinergic-dependent mechanisms of acquisition, expression and reinstatement of nicotine-conditioned place preference by drug priming in rats

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In the present experiments, we employed the conditioned place preference (CPP) paradigm including the establishment, extinction, reinstatement and cross-reinstatement procedures, to study mechanisms of nicotine seeking behavior and interactions between nicotine and morphine. First, we revealed that nicotine produced a place preference to the initially less-preferred compartment paired with its injections during conditioning (0.175 mg/kg, base, *ip*). Once established, nicotine CPP was extinguished by repeated testing. Following this extinction phase, nicotine-experienced rats were challenged with nicotine (0.175 mg/kg, *ip*) or morphine (10 mg/kg, *ip*). These priming injections of both drugs induced a marked preference for the compartment previously paired with nicotine. Furthermore, given the important role of a4b2 nicotinic receptor subtype in the acquisition and maintenance of nicotine dependence, we evaluated and compared the efficacy of varenicline (gift of Pfizer Inc, Groton, USA), a partial a4b2 nicotinic receptor agonist (0.5, 1 and 2 mg/kg, *sc*) and mecamylamine (0.5, 1 and 2 mg/kg, *sc*), a non-selective nicotinic receptor antagonist, in blocking nicotine-induced CPP as well as reinstatement of nicotine CPP provoked by nicotine and morphine The present studies showed the comparable capacity of both nicotinic receptor ligands to attenuate the acquisition and expression of nicotine CPP as well as the expression of reinstatement of nicotine CPP provoked by both drugs. Our results indicated that the cholinergic system, especially through the a4b2 neural nicotinic acetylcholine receptors, plays a pivotal role in the neurobiological processes underlying the relapse to drug addiction, and may suggest that nicotinic receptors could be a potential target for developing effective pharmacotherapy and relapse prevention not only in terms of tobacco smoking in abstinent smokers but also nicotine/opioids co-abuse.

Screening for new $5HT_{1A}$ receptor agonists and antagonists

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5-HT_{1A} receptor has been the subject of several studies since it was shown to be involved in various physiological functions like sleep, appetite and pathological status such as anxiety and depression [Barnes NM and Sharp T, Neuropharmacology, 1999]. In our present study we used similarity-based virtual screening (VS) and VS by docking to identify new 5-HT_{1A} receptor agonists and antagonists. The main steps of VS protocol were ADME/Tox filter, three-dimensional pharmacophore searches and docking protocol. As an input 84 well-known 5-HT_{1A} receptor ligands were used which were subsequently divided into agonists (50) and antagonists (34). In each group several structural classes (arylpiperazine derivatives, tricyclic psychotropic agents, serotonin derivatives etc.) were distinguished. 3D pharmacophores consisting of H-bond acceptor, H-bond donor, hydrophobic aromatic, hydrophobic aliphatic, positive ion and ring aromatic were constructed for both agonists and antagonists. A library consisted of approx. 800 000 drug-like compounds from Enamine Screening Collection has been screened. The library has been sieved with ADME/tox filter consisting of predicted central nervous system activity, octanol/water partition coefficient, apparent Caco-2 cell permeability, brain/blood partition coefficient and human oral absorption (Schrödinger software) followed by pharmacophoric structural elements matching resulting in candidates for agonists and antagonists selection.

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Influence of caffeine on the protective potency of antiepileptic drugs in the 6 Hz psychomotor seizure model in mice

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The aim of this study was to determine the influence of caffeine (administered chronically or acutely) on the protective activity of some newer antiepileptic drugs (AEDs): oxcarbazepine (OXC), levetiracetam (LEV) and lamotrigine (LTG) in the 6 Hz psychomotor seizure model in mice. Caffeine (1,3,7-trimethylxantine) is the most commonly ingested stimulant all over the world. Experimental studies have demonstrated that caffeine, in relatively low (non-convulsive doses), reduces the protective effects of classic antiepileptic drugs against maximal electroshock- and pentylenetetrazol-induced seizures [Kleinrok and Czuczwar, Pharmacol Res, 1990]. The experiments were conducted on male Swiss mice. Topical anaesthetic (0.5% tetracaine hydrochloride) was applied to the cornea before corneal stimulation (0.2 ms duration pulses at 6 Hz for 3 s) administered by constant-current device (ECT Unit 57800; Ugo Basile, Comerio, Italy). The tested drugs and caffeine were administered intraperitoneally. The anticonvulsant activity of the AEDs was determined by evaluating the respective ED50 values, i.e., the calculated doses required to block seizures in 50% of the tested animals. Caffeine administered chronically (twice daily for 15 days) and acutely, at doses 23.1 and 46.2 mg/kg, reduced the protective potency of LEV. That of OXC was decreased by caffeine

administered only chronically at 46.2 mg/kg. However, the anticonvulsant activity of LTG was totally resistant to acute or chronic caffeine. The results indicate that the hazardous effect of caffeine may be extended to another model of experimental epilepsy although not to all AEDs. Because the protective action of LTG is not affected by caffeine in the maximal electroshock-induced convulsions [Chrościńska et al., Pharmacol Rep, 2010] and in the 6 Hz psychomotor seizure model, the untoward interaction of caffeine with LTG in epileptic patients may be not observed.

The effect of chronic co-treatment with memantine and GYKI 52466 on neurotoxicity of dexamethasone – behavioral and histological study

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The long-term treatment with glucocorticoids (GCs) and other preparations e.g. dexamethasone (DEX- a synthetic GCs receptor agonist) or prolonged stress and elevated levels of endogenous corticosteroids are frequently associated with psychosis and cognitive deficits, such as the impairment of memory and learning. GCs potentiate stress or ischemia-induced accumulation of excitatory amino acids (EAA) in the extracellular space of hippocampus. The antagonism of glutamate receptors may play a role in the safe therapy with glucocorticoids. The purpose of this study was to investigate the effect of memantine (non-competitive N-methyl-D-aspartate receptor antagonist) co-administered with the 2.3-benzodiazepine GYKI 52466 (non-competitive AMPA receptor antagonist) on neurotoxic effect of dexamethasone. The experiments were carried out on male Albino Swiss mice (25–30g). Memantine (20 mg/kg) and GYKI 52466 (10 mg/kg) were administered *ip*, 30 and 15 min before DEX (80 mg/kg), *ip* for 7 days. The long-term memory acquisition, motor performance, locomotor activity, as well as body weight and lethality were evaluated 7 days after the drugs administration. Moreover, the morphology of neurons in the CA3 region of the hippocampus in mice was examined. The results of our study have shown that DEX evoked deterioration of all parameters in behavioral tests and damage of neurons in CA3 region of hippocampus. Memantine and GYKI 52466 administered alone for 7 days did not prevent these changes, either. In mice treated with DEX, memantine and GYKI 52466, administered together, improved parameters of behavioral tests and prevented neuronal damage. The above findings suggest that co-treatment with memantine and GYKI 52466 could prevent the neurotoxic effects induced by DEX, but further study needs to be carried out to explain this effect.

Persistent changes in brain monoaminergic systems in neurodevelopmental model of schizophrenia in rats

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We studied the effect of prenatal lipopolysaccharide (LPS) treatment, an animal developmental model of schizophrenia, on brain aminergic system activity in adult offspring. LPS (1 mg/kg) was injected from 7th day of pregnancy, every second day till delivery and resulted in long-lasting schizophrenia-like behavioral changes (enhanced locomotor activity and deficit in prepulse inhibition in their offspring). The levels of DA, 5-HT, NA and their metabolites were measured in male and female 3-months old offspring of dams exposed to LPS or saline. HPLC analysis revealed significant decrease in dopamine and its intracellular metabolite DOPAC in the frontal cortex in male and female rats treated prenatally with LPS. In the striatum the tissue levels of DA were significantly higher in LPS-pretreated female but not in male offspring. There were no statistically significant changes in the level of the extraneuronal metabolite of DA, 3-MT. LPS-pretreated females showed higher 5-HT and its metabolite, 5-HIAA levels in the frontal cortex, whereas in male offspring no changes in these parameters were observed. There was also no changes in the level of 5-HT and 5-HIAA between LPS-pretreated and control animals in the striatum. Additionally, no statistically significant changes in NA concentrations in the frontal cortex between LPS-pretreated and control animals were detected. These data indicate that in the neurodevelopmental model of schizophrenia, higher activity of striatal dopaminergic neurons is connected with an attenuated frontal dopaminergic transmission, which is in accordance with changes observed in schizophrenic patients. Our results showed also that the immune system activation in prenatal period leads to long-term changes in dopaminergic and serotoninergic neuronal activity, associated with schizophrenia-like behavioral disturbances in the offspring.

An involvement of mitogen-activated protein kinase pathway in mechanism of antidepressant drug action

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Some data indicate that levels of mitogen-activated protein kinases (MAPKs) and their phosphatases are disturbed in depression, however the effect of antidepressant drugs on these enzymes is almost completely unexplored. In the present study we investigated the effect of chronic treatment with imipramine, fluoxetine, mirtazapine and tianeptine on extracellular signal-regulated kinases (ERK), Jun N-terminal kinases

(JNK), p38 kinase, MAP kinase phosphatase (MKP-1, MKP-2) and protein phosphatase-2A (PP2A) concentrations in the hippocampus and frontal cortex of control and prenatally stressed rats. It has been found that prenatal stress (an animal model of depression) decreased the levels of active, phosphorylated form of JNK1/2 in the hippocampus and p38 in the frontal cortex, but had no effect on the concentration of p-ERK1/2. The stress-induced decrease in the level of p-p38 in the frontal cortex was attenuated by imipramine, fluoxetine and mirtazapine, whereas the decrease in p-JNK concentration was normalized only by imipramine. Prenatal stress had no effect on MKP-1 and MKP-2 levels whereas the concentration of PP2A was increased in both studied brain structures. Administration of imipramine, fluoxetine, mirtazapine and tianeptine for 3 weeks normalized elevated PP2A level in hippocampus and frontal cortex. The obtained results showed that prenatal stress decreased the levels of active form of JNK and p38, but enhanced PP2A phosphatase expression and most of these changes were reversed by antidepressant drugs. Since JNK and p38 are known to inhibit and PP2A to increase glucocorticoid receptor (GR) function, the changes in their levels may be responsible for enhanced glucocorticoid action in depression. Moreover, these enzymes may be important, intracellular targets for therapeutic action of antidepressant drugs.

Development of ethanol tolerance after chronic free-choice drinking ethanol in the alcohol-preferring WHP rats

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Alcoholism is defined by compulsive, excessive use of alcohol despite negative consequences. Alcohol dependence is usually connected with tolerance to the intoxicating effects of alcohol. Functional or behavioral tolerance is the one of criteria for animal model of alcoholism. The aim of the present study was an evaluation of the development of tolerance during chronic free-choice drinking of ethanol in WHP rats. On day 0, rats were obtained a single intraperitoneal injection of 5.0 g ethanol/kg b.wt. Since the day 0, rats had access to 10% ethanol solution in free-choice condition with the water and food for 12 weeks. Then, rats were once again injected a single IP 5.0 g ethanol/kg b.w. and time of sleep was measured at recovery righting reflex (RR). At recovery RR, blood was drawn from the tail for the determination of alcohol content (BAC). Tolerance was assessed from differences in sleep time on day 0 and sleep time after 12 weeks of chronic free-choice drinking of ethanol and differences in blood alcohol concentrations (BACs) at recovery on day 0 *vs.* 12 weeks of chronic drinking. The mean ethanol intake was 9.14 g/kg/24 h (n = 8) after 12 weeks of chronic consumption of 10% ethanol solution in free-choice conditions with the water. This result revealed that the sleep time of rats exposed to ethanol on day 0 was 203, 55 min *vs*. 146.7 min after 12 weeks of chronic 10% ethanol consumption. The rats exposed to chronic ethanol intake showed shorter sleep time than rats on the day 0. The blood ethanol levels on day 0 was 364.6 mg/dl (n = 8) and 62.01 mg/dl (n = 9) after 12 weeks of chronic 10% ethanol solution intake in two-bottle free condition with the water. WHP rats exposed to alcohol by free-choice drinking exhibited increase alcohol elimination rates after 12 weeks. The studies demonstrate that theWHP rats on chronic free-choice drinking (12 weeks) of alcohol develop metabolic and functional tolerance.

Topiramate attenuates the expression but not the development of tolerance to diazepam-induced motor impairment in mice

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There is evidence that topiramate (TPM), a new antiepileptic drug acting *via* enhancement of GABAergic transmission and blockade of AMPA (but not NMDA) glutamatergic receptors, is effective in various types of addiction [Johnson BA, CNS Drugs, 2005; Sofuoglu M, Psychopharmacology, 2006; Zullino D, Prog Neuropsychopharmacol Biol Psychiatry, 2002]. Among others, the benzodiazepine withdrawal signs were attenuated by TPM [Cheseaux M, Hum Psychopharmacol Clin Exp, 2003]. It is well known that long-term treatment of benzodiazepines leads to development of tolerance to some effects of these drugs (i.e. motor impairment, anticonvulsant action) and to physical dependence [Steppuhn KG, Proc Natl Acad Sci, 1993]. The aim of the present study was to assess the influence of TPM on development and expression of tolerance to diazepam (DZ)-induced motor impairment in mice. Chronic administration of DZ (5 mg/kg) for 10 consecutive days resulted in development of tolerance to motor impairment effect. Motor performance was measured on 1st and 10th day of experiment in rotarod and chimney tests. Pretreatment times were 60 min for TPM (12.5, 25, 50 mg/kg) and 30 min for DZ. The expression of tolerance to DZ-induced motor impairment was significantly inhibited by TPM (12.5, 25, 50 mg/kg) in both tests. However, the development of tolerance to DZ-induced motor impairment was not influenced by TPM. The importance of AMPA receptors antagonism in benzodiazepine withdrawal is pointed in literature data [Cheseaux M, Hum Psychopharmacol Clin Exp, 2003; Steppuhn KG, Proc Natl Acad Sci, 1993]. Then it seems probable that in the attenuated expression of DZ tolerance effects of TPM may participate AMPA receptors. The lack of TPM influence on development of tolerance to DZ-induced motor dysfunction seems to be in agreement with the findings that development of tolerance to DZ in mice can be prevented by concurrent administration of NMDA-antagonist but not of the AMPA-antagonist [Steppuhn KG, Proc Natl Acad Sci, 1993].

Supramolecular organisation of OxPhos complexes changes during ageing in various brain regions to a different extent

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Maintenance and regulation of cellular metabolism depend on activity of numerous reaction pathways which might get affected during ageing. The mammalian brain is very complex. It is presumed that aging occurs differently in different brain areas. We have studied the mitochondrial proteome of the largest brain region, the cortex, and two smaller regions, striatum and hippocampus, of two different age groups, 4–6 and 28 months old rats. Studies of cortex mitochondria showed an age-related decrease in the amount of intact MFoF1 ATP synthase and alteration in its distribution as oligomers, which might be a clue for understanding the link between respiration and longevity. Also the abundance of OxPhos supercomplexes, which are the natural assemblies of the respiratory chain complexes I, III, and IV into supramolecular stoichiometric entities, such as I1III2IV0-4, differs between young and aged cortex tissue. Age-

related changes in the supramolecular architecture of OxPhos complexes could explain alterations in respiratory activity and ROS production during aging. The results observed for the cortex will be discussed and compared to those obtained from hippocampus and striatum. The hippocampus behaves different in many aspects.

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Inhibition of cytosolic phospholipase A2 is involved in the protective effect of nortriptyline on astrocytes exposed to combined oxygen-glucose deprivation

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Protective potential of nortriptyline has been reported in a few experimental models of brain ischemia both *in vivo* and *in vitro*. The main hypothesis concerning cellular mechanism of nortriptyline positive action is that the compound acts as a neuroprotectant presumably through mitochondrial permeability transition (MPT) inhibition, that prevents MPT mediated release of apoptogenic factors, thereby suspending caspase cascade leading to cell death. However, the detailed molecular mechanisms of protective action of the drug are still unresolved. The obtained data imply that the positive effect observed in various animal or cellular models of neurodegeneration is derived from other yet unknown effects or from previously acknowledged mechanisms such as a inhibition of phospholipase A2 (PLA2).

The aim of the present study was to determine whether treatment with the low or medium concentrations of nortriptyline (0.1–10 microM) with proved neuroprotective potential might have an effect on cPLA2 protein and/or mRNA expression in ischemic astrocytes and that this influence might be related to its potential positive influence on cell viability. On the 21[st] day *in vitro*, primary cultures of rat cortical astrocytes were subjected to ischemia-simulating conditions (combined oxygen glucose deprivation, OGD) for 24 h and exposed to nortriptyline. The drug at concentrations of 0.1 and 1 microM attenuated the expression of cPLA2 (both phosphorylated and unphosphorylated form) together with a significant decrease in cPLA2 mRNA level in ischemic astrocytes. We have demonstrated that nortriptyline influences on decrease in cPLA2-mediated arachidonic acid (AA) release through a mechanism which appears to involve attenuation of both PKC and Erk1/2 kinases expression. Nortriptyline also significantly prevented mitochondrial depolarization in ischemic astrocytes. Moreover, the antidepressant protected glial cells against OGD-induced apoptosis and necrosis. Our findings document a role for cPLA2 expression attenuation and AA release inhibition in the protective effect of nortriptyline in ischemic astrocytes.

Effect of adenosine A_{2A} adenosine receptor antagonists on the activity of nigrostriatal DA neurons and catalepsy in rats

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A_{2A} adenosine receptors antagonists are a new non-dopaminergic therapy of Parkinson's disease (PD). The mechanism by which these compounds improve parkinsonian motor dysfunction is related to modulation of the activity of indirect striatopallidal pathway. Presynaptically, adenosine A_{2A} antagonists are able to regulate corticostriatal glutamatergic transmission, while postsynaptically they seem to influence dopamine (DA) synthesis/release, as shown in earlier studies. In the present work, we investigated effect of a new A_{2A} adenosine receptor antagonist KD114 (9-(hydroxyphenethylo)-1,3-dimethylo-6,7,8,9-tetrahydropyrimido[1,2a]purine-2,4(1H,3H)-dione) on DA striatal content, DA release from nigrostriatal terminals and haloperidol-induced catalepsy in rats. It was found that KD114 at doses of 5 and 10 mg/kg, *ip* similarly to KW6002 (3 mg/kg, *ip*) increased DA, DOPAC and HVA tissue level and DA release in the striatum of freely moving animals. However, when given jointly with haloperidol it was not effective in counteracting haloperidol-induced (0.5 mg/kg, *ip*) increase in DOPAC and HVA level in the striatum. KD114 (5 mg/kg) – similarly to other adenosine A_{2A} receptor antagonists (CSC, 1 mg/kg; ZM 241385, 3 mg/kg, KW6002, 3 and 10 mg/kg) – alleviated haloperidol-induced catalepsy. This data allow us to conclude that KD114 is a new promising adenosine A_{2A} receptor antagonist with comparable to other A_{2A} antagonists activity in counteracting motor dysfunction and is able to modulate DA synthesis/release from nigrostriatal neurons.

BDNF/TrkB mRNA expression following antidepressants action in striatal astroglial cells

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In psychopharmacologie of depression we observe two ways of research. One group is focused on catecholamines action. Second one fixes attention on neuronal morphogenesis and synaptic plasticity. Brain-derived neurotrophic factor and its receptor TrkB suppose to coordinate both of above mentioning signaling pathways in depression disturbances. In our experiment we have exploited striatal tissue because in our opinion this structure is misjudge alas in pathophysiology of depression. Several hypothesis proposed striatum us important in future intention activity structure. Real-time PCR analysis was used to determine BDNF/TrkB receptors mRNA expression in cultured striatal astrocytes exposed to different antidepressant drugs. The results demonstrate that chronic ADs administration increase the level of BDNF/TrkB mRNA in examined striatal culture. Our previous study shown that the stimulation of cAMP → CREB pathway after D1 receptors excitation, constitutes a common response to ADs. The present results signify that BDNF/TrkB link it is next neuraltrack (after cAMP/PKA) involved in the CNS adaptation to external conditions altered by chronic ADs treatment. Moreover, the striatum appears to be important formation in antidepressant action thus essential in depression disorder etiology.

Potential antidepressant-like effect of the potent serotonin 5-HT$_6$ receptor agonist EMD 386088 in the forced swim test in rats

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Over the last several years, the 5-HT$_6$ receptor has emerged as a highly interesting molecular target which thoroughly interacts with antidepressant drugs. A question comes up whether the potential therapeutic indication, i.e. depression will be best served by agonists or antagonists of this receptor, since equivalent antidepressant potency and efficacy can be delivered in animal models by both kind of 5-HT$_6$ receptor ligands. More studies so far have concentrated on effects provided by selective 5-HT$_6$ receptor antagonists [e.g. Hirano, Life Sci, 2009; Wesołowska, Pharmacol Rep, 2010]. Data concerning potential antidepressant activity of 5-HT$_6$ agonists are sparse and incomplete. EMD 386088 is a potent 5-HT$_6$ receptor agonist (EC50 = 1.0 nM) that displays selectivity over other serotonin receptors (IC50 = 7.4, 110, 180, 240, 450, 620, 660 and 3000 nM for 5-HT$_6$, 5-HT$_{1D}$, 5-HT$_{1B}$, 5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_4$, 5-HT$_{1A}$ and 5-HT$_7$ receptors, respectively). Moreover, it shows moderate affinity at 5-HT$_3$ receptors (IC50 = 34 nM) [Mattsson, Bioorg Med Chem Lett, 2005]. In the present study, we examined the potency of EMD 386088 to inhibit the immobility time of Wistar rats in the forced swim test. Antidepressant drugs, imipramine and citalopram were tested for comparison. EMD 386088 (10 mg/kg), given *ip*, both 30 and 60 min before the experiment, significantly decreased immobility time of rats. It produced more distinct effect after administration 30 min before the test. Moreover, its effect seems to be specific, since EMD 386088 (10 mg/kg) did not increase any parameters of the exploratory activity of rats measured in the automated version of the open field. As expected, imipramine (30 mg/kg, but not 20 mg/kg) produced antidepressant-like activity in the same procedure. Citalopram (20–30 mg/kg) did not induce any effects characteristic of antidepressants in that model. To the best of our knowledge, the present results are the first preclinical report indicating that EMD 386088 may have antidepressant-like activity detecting in the forced swim test in rats.

Toxic fragment of alpha-synuclein, NAC peptide induce apoptotic cell death by activation of p53/Cdk5 signalling pathway

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The non-Abeta component (NAC) of Alzheimer's disease (AD) has been identified as the second major constituent of the senile plaques in AD brains. This 35-residue peptide is derived from a larger precursor protein NACP/alpha-synuclein. The molecular mechanisms of NAC and alpha-synuclein toxicity is not fully understood. Our previous study presented that both full-length alpha-synuclein and its neurotoxic

fragment NAC induced oxidative/nitrosative stress. Moreover, it was shown that NAC increased the translocation of nuclear factor kappa B (NFkB) and its activity, that subsequently may influence expression of several genes, including Tp53. Our present study focused on the role of p53 protein in mechanism of PC12 cells death evoked by NAC peptide. Here we found that exposure of PC12 cells to exogenous NAC peptide (10 microM) enhanced free radical generation, induced mitochondria dysfunction and cell death. We also observed time-dependent enhancement of Tp53 gene expression after NAC treatment. The inhibition of p53 by pifithrin significantly protected PC12 cells against NAC peptide-evoked mitochondria failure and death. In addition, exposure to NAC peptide resulted in the higher expression of cyclin-dependent kinase 5 (Cdk5), one of the enzymes responsible for p53 phosphorylation and activation. Concomitantly, we observed the increase of expression of Cdk5r1 and Cdk5r2 genes, coding p35 and p39 peptides, that are essential co-factors in regulation of Cdk5 activity. Moreover, the specific Cdk5 inhibitor (BML-259, 10 microM) protected large population of cells against NAC-evoked cell death. Our findings indicate that NAC peptide exerts its toxic effect by activation of p53/Cdk5-dependent apoptotic signaling pathway. This new experimental paradigm will help in developing therapeutic strategy in AD and might contribute to the understanding of the area of overlap between toxic effect of different amyloid peptides.

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Effect of chronically administrated antiepileptic drugs on brain production of kynurenic acid – study *in vivo*

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Kynurenic acid (KYNA) is an endogenous brain constituent which inhibits the activity of three ionotropic amino acid receptors (EAA). KYNA displays the highest affinity towards the glycine site of the NMDA receptors complex. The cerebral synthesis of KYNA from its bioprecursor L-kynurenine is catalyzed by two distinct kynurenine aminotransferases (KAT I and KAT II). KYNA displays anticonvulsant and neuroprotective effects *in vivo* and *in vitro* and its altered metabolism was implicated in the pathogenesis in the pathogenesis of seizures. Our previous data indicate that acute application of some antiepileptic drugs *in vitro* may increase KYNA production. Here, the chronic effect of phenobarbital and phenytoine on level of KYNA and the activites of KAT I and KAT II was studied in rats. The animals were administered phenobarbital or phenytoine (10 mg/kg, *ip* and 25 mg/kg, *ip* respectively) for 14 days. Brain cortex was collected 24 hrs after the last injection of a drug and the level of KYNA and activities (KATs) I and II were assessed. KYNA was quantified using HPLC system with fluorometric detector. Long-term phenobarbital administration significantly increased level of KYNA as well as the avtivities of KAT I and II to 136%; 152% and 164% of control, respectively. Similarly, chronic treatment of phenytoine increased KYNA levels and the activities KAT I and II to 214%; 196% and 319% of control, respectively. Presented results suggest that chronic therapy with phenobarbital and phenytoine might increase level of kynurenic acid in the brain what may contribute to their antiepileptic effects.

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The effect of $5HT_{1B}$ receptor ligands on amphetamine self-administration in rats

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Besides cocaine, amphetamine belongs to psychostimulants that shows distinct rewarding properties, which determines its dependence-evoking action. This and other behavioral effects of amphetamine are connected with activation of brain dopaminergic systems, being a consequence of dopamine (DA)-releasing activity and the inhibitory action on DA reuptake and MAO A (an enzyme responsible for DA catabolism) activity [Seiden and Sobol, Ann Rev Pharmacol Toxicol, 1993; Fleckenstein et al., Ann Rev Pharmacol Toxicol, 2007].

Apart from DA, amphetamine also releases other neurotransmitters, especially noradrenaline and 5-hydroxytryptamine (5-HT) [Muller et al., Prog Neurobiol, 2007]. Since we have recently gathered evidence that 5-HT_{1B} receptor ligands modify the rewarding properties of cocaine [Przegaliński at al., Eur J Pharmacol, 2007; Pharmacol Rep, 2008] we undertook studies into the effects of such ligands on the rewarding properties of amphetamine in self-administration model in rats. Male Wistar rats were trained to self-administer amphetamine (15–120 mg/kg/injection) and were systematically pretreated with the selective 5HT1B receptor antagonist SB 216641 or agonist (CP94253) before a test session during the maintenance phase. The dose-response curve of amphetamine was bell-shaped, the maximum effect being observed after a dose of 60 mg/kg/injection of the psychostimulant. SB 216641 (2.5–5 mg/kg, *ip*) was inactive in altering amphetamine self-administration (60–120 mg/kg/injection) on the descending limb of its dose-effect function. On the other hand, CP 94253 (2.5–5 mg/kg, *ip*) attenuated amphetamine (60–120 mg/kg/ injection) self-administration in a manner similar to that produced by an increased unit dose of amphetamine. Our findings indicate that tonic activation of $5HT_{1B}$ receptors is not essential to rewarding properties of amphetamine, whereas pharmacological stimulation of 5-HT_{1B} receptors enhances the latter property of the psychostimulant.

Effects of the H3 receptor antagonist ABT239 on nicotine-induced sensitization and memory enhancement in rodents

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Nicotine, the main psychoactive ingredient in tobacco appears responsible for smoking reinforcement [Zaniewska et al., Pharmacol Rep, 2009]. The addictive potential of nicotine (Nic) is linked to mood-modulating and cognition-enhancing effects and the learning and memory processes in the development of the addictive properties of Nic and other abused drugs have been discussed. Histamine (H)3 receptor antagonism has similarly received attention for it's role in cognition [Esbenshade et al., Br J Pharmacol, 2008]. Modulation of the associative learning processes during the development of addiction might impact the addictive process [von der Goltz and Kiefer, Eur Arch Psychiatry Clin Neurosci, 2009]. We therefore investigate the role of H3 antagonist (ABT239) *vs*. Nic interactions on Nic sensitization/conditioned locomotion

in rats and memory-related responses relevant for addiction in mice. ABT239 (0.3–3 mg/kg) did not alter basal, Nic-evoked (0.4 mg/kg) locomotor responses, expression of sensitization, or cue-conditioned locomotion. However, rats pretreated with a separate dose of ABT239 (1 mg/kg) prior to Nic (0.4 mg/kg) for 5 days and then challenged with Nic (0.4 mg/kg) after a 5-day withdrawal period, showed significantly higher locomotor hyperactivity in comparison with the effect observed in Nic-pretreated and Nic challenged rats. In mice, acute administration of ABT239 (3 mg/kg) or Nic (0.035 mg/kg), in combination, reduced, in the acquisition phase, transfer latency (open-closed arms), while ABT239 (0.3–3 mg/kg) or Nic (0.035 mg/kg) produced similar effects in the consolidation phase. In combination studies ABT239 (0.1 mg/kg) and Nic (0.0175 mg/kg) induced significant improvement in both acquisition and consolidation. Our findings implicate H3 and Nic interactions in the acquisition and consolidation of memory, however more studies will be needed to fully understand these interactions. A role for H3 receptors in Nic sensitization seems limited.

Impairment in pain perception in rats with central serotoninergic system lesion

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The aim of the present study was to examine the effect of the central serotoninergic lesion on the antinociceptive effects of morphine (5.0 mg/kg), nefopam (20 mg/kg), indomethacin (5.0 mg/kg) and imipramine (10 mg/kg) in the models of exteroceptive sensation (tail immersion, paw pressure and formalin tests and interoceptive sensation (writhing test). On the 3rd day of postnatal life male rats were administered with 5.7-DHT (70 µg/10 µl in 0.1% ascorbic acid solutions ICV; 35 µg/5 µl per side); control animals received vehicle (70 µg/10 µl of 0.1% ascorbic acid solutions ICV). Rats continued to be housed until 8–10 weeks, for further experimentation. It was shown that the central serotoninergic lesion did not affect antinociceptive effects of morphine, nefopam, indomethacin and imipramine assessed in tail immersion and paw pressure tests. At the same time it was demonstrated that the destruction of serotoninergic nerves slightly modified analgesia evoked by nefopam and indomethacin in formalin test but much more profound reduction in analgesia produced by morphine and indomethacin injection was observed in visceral model of nociception. In biochemical study it was shown that analgetics employed in this study (with exception of indomethacin) altered synthesis rate of serotonin, norepinephrine and dopamine in the examined parts of the rats brain. The above indicates that the monoaminergic systems (serotoninergic, noradrenergic and dopaminergic) participate in the central mechanism of action of these drugs. It is likely that similar nociception abnormalities may occur in patients with serotoninergic system dysfunction, so that it points out on the requirement of analgetics dosage adjustment.

Acamprosate attenuates anxiety-like behavior during amphetamine withdrawal in rats

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The process of addiction is thought to be maintained by negative affective symptoms that are strongly expressed after cessation of drug taking. An increase in depression and anxiety-like behaviors manifested after chronic administration of amphetamine certainly contribute to drug relapse. Therefore, examination of the behavioral effects of amphetamine withdrawal in rodents may provide insights into the neurobiological mechanisms underlying post drug anxiety. It is also well known that glutamate play a major role in anxiety. Acamprosate, the glutamatergic neurotransmitter system modulator, is considered safe and effective in the maintenance of abstinence in patients with alcohol dependence. Memantine is a low-affinity N-methyl-D-aspartate receptor antagonist approved in Alzheimer's disease treatment. We hypothesized that these compounds may also be effective in psychostimulant withdrawal. To test this hypothesis, an involvement of glutamatergic neurotransmission in amphetamine withdrawal symptoms was evaluated 24 h after 14 days of constant dose amphetamine administration (2.5 mg/kg daily) in male Wistar rats. One day withdrawal from chronic administration of amphetamine resulted in an increased anxiety-like behavior measured by the elevated plus maze and no change in locomotor activity of animals was observed. These behavioral effects were dose-dependently reduced by acute administration of acamprosate (200 and 400 mg/kg). On the other hand, memantine did not change anxiety-like effects of amphetamine withdrawal. Our results demonstrate that early withdrawal from amphetamine is accompanied by increased anxiety, and this state was reversed by modulation of glutamatergic neurotransmission by acamprosate.

Influence of naltrexone on pain sensitiveness and paw edema during chronic inflammation in animals under normal or stress conditions

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There is growing body of scientific evidence indicating multidirectional, health promoting effects of low dose naltrexone (NTX) therapy. Increased endogenous opioid activity and modulation of immunological system induced by low dose NTX may possibly be concerned with attenuation of diverse oncogenic processes and autoimmune reactions. Our study were aimed at the evaluation of the NTX effect on paw edema and pain sensitiveness in the animal model of chronic inflammation under normal or stress conditions. Experiments were performed on male Wistar rats. Complete Freund's Adjuwant (CFA; 0.15 ml) was injected into the plantar side of the left hind paw. Paw volume and pain sensitiveness was evaluated before, one day after and then in 3 day intervals during 14 days from CFA injection. Paw volume was measured by pletysmometer and pain sensitiveness using electronic von Frey apparatus. As from the day of CFA application NTX was daily injected in the 0.1; 0.5 and 1 mg/kg dose both in stressed and non-

stressed animals. Stress conditions were evoked by 5-minute daily swimming in water at temperature 21 ± 0.5°C starting from CFA application.

CFA induced significant increase in paw volume (~60%) and pain sensitiveness (~65%). During two-week observation both parameters markedly and similarly gradually grew both in normal and stress conditions. In non-stressed animals NTX administration in all doses prevented subsequent significant increase of paw volume from the initial rise. In stressed rats NTX in 0.1 and 0.5 mg/kg dose did not prevent, but in the 1 mg/kg dose even decreased (by 9.2 %) paw volume in the last measurement in comparison to the first one. NTX in all studied doses prevented subsequent marked increase in pain sensitiveness both in normal and stress conditions. Application of low dose NTX in conditions with chronic inflammatory and pain states could improve therapy outcomes, reduce analgesic and anti-inflammatory drugs intake and consequently their side effects.

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Desipramine increased experimental lung tumor growth in stress-sensitive rats in the chronic mild stress model of depression

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In almost all world lung cancer is the most common cause of cancer related deaths in males. In Europe, lung cancer accounts for about 25% of deaths from cancer among the men and 9% among women. Several scientific clinical and experimental studies confirmed the role of psychosocial factors (e.g. stress) and personality traits not only in developing but also in progress of cancer process. The effect of antidepressant drugs on tumor progress in experimental models of stress is very poorly recognized. In present study we established a role of susceptibility to stress in modulatory effect of antidepressant drug on tumor growth. The aim of present study was to study the effect of individual reactivity to stress and two weeks desipramine administration after injection of MADB 106 cells on their metastatic colonization in lungs of male Wistar rats. Rats were subjected by three weeks to chronic mild stress (CMS) model of depression and high-reactive and non-reactive rats were selected on the base of sweet-solution preference-test. All selected animals received *iv* tumor cells and were further subdivided: for two additional weeks the rats received daily *ip* injection of desipramine (10 mg/kg) or saline. Chronic desipramine treatment significantly increased number of lung metastasis in both stress high reactive and no-reactive rates in comparison to saline treated control rats. Following intravenous injection MADB 106 tumor cells metastasize only to the lungs and immune-dependent the lung clearance is limited to the first 24 h after tumor cells injection so it is why immune cell subpopulations and natural killer (NK) cell activity were studied 24-h after single desipramine injection in stress high reactive and no-reactive rates. We may suggested that increase in lung metastasis was connected with inhibitory effect of desipramine injection on number of TCD8+ and B cells but not NK cell activity.

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The role of the cerebellar catecholaminergic systems in the harmaline-induced tremor in rats

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Activation of the glutamatergic olivo-cerebellar pathway has been suggested to be crucial for the harmaline-induced tremor. However, this symptom seems to be additionally influenced by 5-HT, noradrenergic (NA) and dopaminergic (DA) systems. The aim of the present study was to examine a contribution of the cerebellar catecholaminergic innervations to the harmaline-induced tremor in rats. Rats were injected bilaterally into the cerebellar vermis with 6-OHDA (8 mg/0.5 ml) either alone or in combination with desipramine (DMI, 15 mg/kg, *ip*). Harmaline was administered to animals in doses of 7.5 or 15 mg/kg, *ip*. Tremor of forelimbs was measured as a number of episodes during the 90-min observation. Rats were killed by decapitation 30 or 120 min after harmaline. The levels of DA, NA, 5-HT and their metabolites were measured by HPLC in the cerebellum, striatum and frontal cortex. 6-OHDA injected alone induced ca. 40–80% decreases in NA level in the cerebellum. Furthermore, the above lesion enhanced the harmaline-induced tremor of forelimbs. 6-OHDA + DMI decreased DA transmission in some regions of the cerebellum while inducing its compensatory activation in others. The latter lesion did not markedly influence the tremor induced by harmaline. Harmaline decreased the MAO-dependent metabolism of all monoamines and induced increases in their levels in the striatum, frontal cortex and/or cerebellum. 6-OHDA-induced lesion of the NA system prevented the harmaline-induced increase in NA level in the cerebellum, and further enhanced 5-HT system activation induced by this compound in the striatum and frontal cortex. The present study indicates that NA innervation of the cerebellum interacts with 5-HT cerebral systems and plays an inhibitory role in the harmaline-induced tremor.

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Differences in the density of GABA(A)R alpha-2 subunit and gephyrin in the brain structures of low and high anxiety rats in basal and fear-stimulated conditions

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In this study we used a newly designed model of individual differences in fear responses in rats selected according to their behavior in the contextual fear test (i.e. the duration of a freezing response was used as a discriminating variable): low responders (LR) were defined as rats with a duration of freezing response one standard error or more below the mean value, and high responders (HR) were defined as rats with a duration of freezing response one standard error or more above the mean value. We assessed the differences in

the density of GABA(A) receptor alpha-2 subunits and gephyrin in the brain structures of low (LR) and high (HR) anxiety rats subjected to extinction trials and re-learning of a conditioned fear response. An increased basal concentration of alpha-2 subunit in the basolateral amygdala (BLA) was found in HR rats (western blot and immunocytochemistry), and the density of alpha-2 subunit in the basolateral amygdala negatively correlated with the duration of freezing responses in the aversive context, in the same group of rats. This finding supports previous data on the role of GABA(A) receptor alpha-2 subunit in the BLA in the expression of anxiety-like behavior. An exposure of HR animals to fear conditioned context on re-test of conditioned fear test increased in the BLA, prefrontal cortex and hippocampus (dentate gyrus) of HR animals, the expression of alpha-2 subunit and gephyrin, and these phenomena were accompanied by a significantly slower rate of extinction of conditioned fear responses. These findings suggest, that animals more vulnerable to stress might have deficits in the innate regulation of the intracellular mechanisms controlling the trafficking of GABA(A) receptors in the limbic structures (hippocampus and amygdala), involved in the control of emotional behavior. These data indicate also a possible mechanism of individual differences in the effects of benzodiazepines found among patients with anxiety disorders.

The gut-brain barrier in major depression

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In depression takes part inflammatory response characterized by an activation of cell-mediated immunity, i.e. a T lymphocytic and monocytic activation. This theory, called the monocyte–T lymphocyte, cytokine or inflammatory hypothesis of depression posits that an increased production of T lymphocytic, e.g. interferon gamma (IFNγ) and interleukin-2 (IL-2), and monocytic cytokines, such as IL-1β, IL-6, and tumor necrosis factor alpha (TNFα) induces depressive symptoms, disorders in the serotonergic system and an acute phase response. Since then many reports have been published on increased levels of proinflammatory cytokines and acute phase proteins in depression. In animal models, induction of a peripheral inflammation, for example by lipopolisaccharide (LPS) leads to a central neuroinflammation accompanied by depressive symptoms. In humans, cytokine-based immunotherapy induces depressive symptoms, which are closely related to the induction of the cytokine network and changes in the metabolism of serotonin. This hypothesis also considers the fact that psychosocial factors contribute to depression, since psychosocial stress has been shown to induce inflammatory pathway. The aim of present study was to examine whether an increased of gastrointestinal permability with an increased translocation of LPS from gram negative bacteria may play role in the pathophysiology of depression. Toward this end, the present study examines the serum concentrations of IgM and IgA against LPS of the gram negative bacteria. We found that the prevalences and median values for serum IgM and IgA against LPS of enterobacteria are significantly greater in patients with depression than in normal volunteers. The symptom profiles of increased IgM and IgA levels are fatigue, autonomic and gastrointestinal symptoms and subjective feeling of infection. The results show that intestinal mucosal dysfunction characterized by an increased translocation of gram-negative bacteria (leaky gut) plays a role in the inflammatory pathophisiology of depression.

The effect of simulated ischemia, N-acetylcysteine, ebselen and glutamic acid on signaling kinases and structural proteins of rat brain endothelial cells *in vitro*

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We analyzed the expression of signaling kinases and specific structural proteins in rat's brain endothelial cells cultured *in vitro* and exposed to simulated ischemic conditions (OGD, oxygen-glucose deprivation). The effects of ebselen, N-acetylcysteine and glutamic acid were also evaluated. The fragments of rat brain microvessels 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. OGD was obtained by incubation of cells in humidified atmosphere (3% O_2, 92% N_2, 5% CO_2) in medium without glucose and serum. Confluent cultures of endothelial cells were exposed to: 5,000 μM glutamic acid, 200 μM N-acetylcysteine or 20 μM ebselen. Intracellular signaling kinases (Akt, ERK1/2) and structural proteins (VE-cadherin, occludin, claudin, MDR-1 and JAM-1) were detected using Western-blot. OGD significantly elevated ERK1/2 and decreased AKT expression as well as JAM-1, occludine, claudin and MDR-1. N-acetylcysteine increased JAM-1 level in normoxia and OGD and diminished MDR-1 level in OGD. Ebselen decreased VE-cadherin, JAM-1 and MDR-1 level in ischemia. Moreover, ebselen diminished MDR-1 expression in OGD. Glutamic acid increased JAM-1 in OGD and MDR-1 level in both conditions. OGD affected the expression of signaling kinases. Glutamic acid exerted combined effect on the level of specific proteins of endothelial cells. Ebselen in OGD possesed rather inhibitory effect on those proteins' expression.

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The effect of acute and chronic salsolinol administration on L-DOPA-induced changes in monoamine metabolism in rat brain structures

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Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoine) aroused a considerable interest as substance that may be implicated in etiology of Parkinson's disease (PD). A compound may be regarded both as environmental and endogenous neurotoxin. In a clinical study we have found that the concentration of salsolinol in the cerebrospinal fluid of patients with advanced parkinsonism was significantly augmented, and this increase is related to the state of dementia rather than of advancement of parkinsonism [Antkiewicz-Michaluk et al., Biol Psychiatry, 1997]. L-DOPA is the main medication used for the treatment of Parkinson's disease, and in this study we evaluated the effects of single and multiple salsolinol administration on L-DOPA- induced changes in the rate of monoamines metabolism in different structures of rat brain. The experiments were carried out on male Wistar rats weighing 250–280 g. The biochemi-

cal data: the concentration of dopamine (DA), noradrenaline (NA) and serotonin (5-HT) and their metabolites were established by HPLC methodology with electrochemical detection.

Results: the experiments have shown that both acute and multiple administration of salsolinol in investigated doses (50 and 100 mg/kg, *ip*) did not change the rate of monoamines metabolism in rat brain structures. L-DOPA (100 mg/kg, *ip* + Carbidopa 10 mg/kg, *ip*) produced a strong increase of dopamine, noradrenaline and serotonin metabolism in rat brain structures. Chronic (14 days)but not acute administration of salsolinol (100 mg/kg, *ip*) produced long lasting (up to 24 hr) antagonism to L-DOPA-induced an increase of dopamine concentration and activation of the rate of dopamine and serotonin metabolism in striatum and frontal cortex in rat. Opposite to that, the effect of L-DOPA on the rate of noradrenaline metabolism was much stronger inhibited by acute administration of salsolinol, and nearly not change by its multiple treatment.

Conclusion: the present results demonstrated that chronic administration of salsolinol impaired L-DOPA-produced activation of DA and 5-HT system in rat striatum. Additionally, these results are also important from clinical point of view because suggest that concentration of endogenous salsolinol in the brain of parkinsonian patients may be an important factor of efficacy of L-DOPA therapy.

Effect of kudzu (*Pueraria lobata*) on ghrelin blood level in alcohol Warsaw High Preferring and Warsaw Low Preferring rats

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There is a hypothesis that ghrelin, which is able to induce appetite, may contribute to the development of the dependence and craving through long-term alcohol use [Addolorato et al., Drug Alcohol Rev, 2009]. It is known, that ghrelin serum levels were found to be altered in alcohol-dependent patients and central ghrelin administration (to brain ventricles or to tegmental areas involved in reward) increased alcohol intake in a 2-bottle (alcohol/water) paradigm in mice [Jerlhag et al., PNAS, 2009]. The aim of this study was to assess the effect of an extract from *Pueraria lobata* root (kudzu) (KU), which is involved in reducing alcohol intake in experimental animals [Keung, Med Res Rev, 2003], on ghrelin plasma in the model of alcoholism. The experiments were performed on Warsaw High Preferring (WHP) and Warsaw Low Preferring (WLP) of male rats received 10% ethanol using voluntary intake procedure for 4 weeks. Alcohol-naive WHP and WLP rats were used as controls. Next, both ethanol-drinking and ethanol-naive rats were treated with water extract of KU (500 mg/kg, *po*) for 21 consecutive days and acylated ghrelin (active form) levels in plasma were measured using ELISA method. KU treatment lowered alcohol intake in WHP animals (75%), but not that of WLP rats. It was found that plasma ghrelin concentrations in ethanol-naive WHP animals showed a significantly higher level (68%) when compared with the ethanol-naive WLP rats. Ethanol consumption in WHP rats resulted in significant reduction of the peptide concentration (32%) while in WLP animals alcohol did not affect the ghrelin concentration. KU administra-

tion lowered ghrelin levels both in ethanol-drinking (41%) and ethanol-naive WHP rats (37%), whereas in WLP rats KU did not change the peptide levels. In conclusion, it was found out that ghrelin can be a marker both for ethanol craving and intake, whereas effect of KU on ghrelin level is probably not coupled with ethanol activity.

Zinc deficiency-induced pro-depressive behavior in mice. Correlation with corticosterone concentration

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Zinc is one of the most important trace element in live organisms. Its deficiency retards the growth of humans and animals and also affects brain function. There are some data suggesting presence of link between zinc and depression. Up to 50% of the population is thought to have inadequate levels of zinc in their blood. Zinc shows antidepressant-like properties in tests and models of depression. Zinc augments antidepressant therapy in human depression. It is still unknown if low zinc levels lead to development of depression or whether zinc deficiency is a result of depression. The aim of the study was to assess if lack of this trace element contribute to development of pro-depressive behavior. Mice behavior was assessed after 2, 4 and 10 weeks of zinc deficient diet. To evaluate animal antidepressant-like activity, we used tail suspension test and forced swim test. After 2-weeks of zinc deprivation, a significant reduction in immobility time was noted in both tests. After 4- and 10-week zinc deficiency, mice demonstrated pro-depressive behavior (increased immobility time). There were significant enhancement in serum corticosterone level in mice after 4 and 10, but not 2-week of administration of zinc deficient diet. The time course of the zinc deficiency-induced pro-depressive behavior is correlated with hyperactivity of HPA axis common in this mood disorder. In conclusion, these preclinical studies indicate to zinc deficiency as a possible cause of depression.

Effect of prenatal manganese intoxication on the neurotoxic action of 6-hydroxydopamine in rats

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The present study was designed to explore the role of ontogenetic manganese (Mn^{2+}) exposure on a neurotoxic potential of 6-hydroxydopamine (6-OHDA) in the dopaminergic pathway in rats. Pregnant Wistar rats were given tap water containing $MnCl_2$ (10,000 ppm) for the duration of pregnancy and lactation. Regular tap water was substituted at weaning. Control rats were derived from dams that consumed tap water

throughout pregnancy, and had no exposure to Mn^{2+} afterwards. At 3 days after birth animals of both tested groups were given bilateral *icv* injection of 6-OHDA HBr in one of three doses (15 μg, 30 μg or 67 μg base form on each side), or vehicle saline (control). 6-OHDA lesioned and control rats were housed until 8–10 weeks of age for further experiments. By means of dopamine receptor agonist and antagonist administration characteristic behavior as episodes of chewing and yawning, stereotypy and catalepsy were evoked in laboratory animals. Besides, content of biogenic amines and metabolites as well as dopamine synthesis rate in some parts of the brain were assayed. It was demonstrated that manganese as an additional neurotoxic factor has accelerated disclosure of dopamine D2 receptor supersensitivity in rats with mild dopamine lesion (2 × 15 μg of 6-OHDA). Biochemical studies revealed decrease in dopamine and its metabolites in frontal cortex and hippocampus. Findings from the present study demonstrate that ontogenetic manganese exposure intensifies neurotoxic action of 6-OHDA on dopaminergic system in rats. Obtained results provide evidence that manganese intoxication can be one of environmental factor predisposing to neurodegenerative disorders development.

Some behavioral effects of carbamazepine and olanzapine in rats

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As shown by clinical studies, combinations of first generation normothymics (carbamazepine – CBZ) with atypical neuroleptics (olanzapine – OLA) leads to an improvement in approximately half of patients treated for relapses of the bipolar affective disease.

At the same time, in patients treated for bipolar affective disease or schizophrenia a deterioration of cognitive functions, in particular memory is observed. It has been found that cognitive function deficits in bipolar affective disease or schizophrenia may be mitigated with pharmacotherapy, although it should be noted that some drugs (typical neuroleptics) do not improve cognitive skills. The present study was conducted to investigate the efficacy of combined use of OLA with CBZ on spatial memory functions in the Morris test. We also detemined the influence of these drugs on antidepressant effect in the Porsolt test. Considering the fact the new normothymic drugs, unlike typical neuroleptics, less frequently induce side effects, it was important to examine any adverse effects resulting from combined administration of OLA and CBZ measured in the chimney test (motor coordination).

Chronic co-administration of OLA and CBZ improves spatial memory functions. Antidepressant effect was observed after single and 7-days' co-administration of these drugs. Disturbance of motor coordination was observed only after single co-administration of drugs. Co-administration of OLA and CBZ can be useful in mental and psychiatric diseases with memory function disorders.

Effect of fluoxetine on interleukin-1 beta in rat brain – *in vivo* and *in vitro* studies

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Results of experimental studies especially *in vitro* studies and studies concerning peripheral cytokines indicate that some of antidepressants down-regulate production of pro-inflammatory cytokines. Our experiments were designed to determine if chronic treatment with fluoxetine (FLX), a selective inhibitor of serotonin reuptake, affects lipopolysaccharide (LPS)-stimulated IL-1 beta in brain structures, and whether this antidepressant has influence on IL-1beta production by glial cells. FLX (10 mg/kg/24 h) was administered orally to male Spraque-Dawley rats for 21 days in 2% saccharin solution. On day 22, LPS was injected (250 μg/kg, *ip*) 2 h before decapitation. Brain structures such as pituitary, hypothalamus, hippocampus and frontal cortex were dissected out. IL-1 beta concentration was measured by using ELISA kits (R&D Systems). Protein concentration was determined by Bradford's method. In the pituitary, the level of IL-1beta mRNA was evaluated by QRT-PCR assay using Applied Biosystems reagents. *In vitro* studies were carried out on 13–14 day primary rat mixed glial cultures stimulated by LPS (2 μg/ml) for 48 h. IL-1beta mRNA and NFκB p65 subunit levels (Millipore) in cell nucleus fraction were determined. A tendency to an increase in the LPS-stimulated IL-1beta concentration in the pituitary and hypothalamus (about 25%) was observed. Similarly, FLX given chronically non-significantly increased LPS-induced IL-1beta mRNA in the pituitary. In opposite, in glia culture FLX in the dose from 10^{-6} to 10 μM diminished LPS-stimulated IL-1beta release. FLX at concentration of 10 μM reduced the level of IL-1beta mRNA and NF Bp65 levels. Our results suggest that FLX directly modulates glial cells activity but when given *in vivo* does not exert anti-inflammatory effect.

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Evaluation of anxiolytic-like activity of zinc

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Zinc exhibits antidepressant-like activity in preclinical tests (the forced swim test and tail suspension test) and in olfactory bulbectomy, chronic unpredictable stress and chronic mild stress; three models of depression. Zinc also enhances the efficacy of pharmacotherapy of depression in humans [Siwek et al., J Affect Disord, 2009]. In the present study we evaluated the anxiolytic-like activity of zinc hydroaspartate in plus-maze test in rats after acute intraperitoneal injection. In these test zinc hydroaspartate was administered *ip* at a doses of 65 and 32.5 mg/kg. Anxiolytic drug, diazepam, was tested for comparison. Zinc given at a dose of 32.5 mg/kg significantly increased (up to 57%) the percentage of time spent in the open arms. The percentage of entries into the open arms did not reach the level of statistical significance, however, a 35% increase of entries into

the open arms was observed. Higher dose of compound (65 mg/kg) did not demonstrate any effects characteristic of anxiolytics in that test, while this dose decreased the locomotor activity of rats.

The above data demonstrate that zinc exhibits anxiolytic-like effect in the animal test and may suggest such activity in human disorders.

Interaction of glycine/NMDA receptor ligands and antidepressant drugs in the forced swim test

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The influence of two glycine/NMDA receptor ligands, namely L-701,324 (antagonist) and D-cycloserine (partial agonist) on the action of antidepressant drugs with different pharmacological profiles fluoxetine, reboxetine and tianeptine, was investigated in the forced swim test (FST) in mice. L-701,324 and D-cycloserine in ineffective doses (1 mg/kg and 2.5 mg/kg, respectively), administered concomitantly with reboxetine (2.5 mg/kg), did not change the behavior of animals in the FST. A synergistic effect was seen when both glycine/NMDA receptor ligands were given jointly with fluoxetine (5 mg/kg) or tianeptine (20 mg/kg) in the FST without significant changes in locomotor activity. The antidepressant-like activity of all the used antidepressant drugs was abolished by D-serine co-treatment. Moreover, the antidepressant-like action of L-701,324 (4 mg/kg) and D-cycloserine (5 mg/kg) was significantly reduced by pretreatment of mice with an inhibitor of serotonin synthesis, p-chlorophenylalanine. However, a selective depletion of noradrenaline pathway induced by DSP-4 did not change antidepressant action of both drugs. Thus, the antidepressant-like action of the glycine/NMDA ligands seems to involve serotonergic system.

Involvement of the serotonergic and glutamatergic systems in the antidepressant-like activity of MTEP, in the forced swim test in mice

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Several lines of evidence suggest an antidepressant-like activity of highly selective, noncompetitive antagonist of mGluR5 receptors 3-[(methyl-1,3-thiazol-4-yl)ethynyl]-pyridine (MTEP) after acute and chronic treatments in behavioral tests and in experimental models of depression (the forced swim test, the tail

suspension test, oflactory bulbectomy model of depression) [Pałucha et. al., Drug News Perspect, 2005; Pilc et. al., Neuropharmacology, 2002]. Mechanism of action of mGluR5 antagonists is not yet well elucidated. The aim of this study was to investigate whether the antidepressant-like action of is dependent upon serotoninergic and glutamatergic systems. The experiment was carried out on male Albino Swiss mice and to detect antidepressant-like activity the forced swim test (FST) was employed. MTEP administrated at doses of 0.3, 1 or 3 mg/kg (*ip*), 45 min before test, decreased the immobility time in the FST. More specifically, the dose of 0.3 mg/kg reduced the immobility time by 30 % ($p < 0.001$). None of the used doses affected the spontaneous locomotor activity. Antidepressant-like effect of MTEP (0.3 mg/kg) was significantly ($p < 0.001$) blocked by pretreatment with the 5HT-2A/2C receptor antagonist ritanserin (4 mg/kg, *ip*) while the 5HT-1A receptor antagonist WAY 1006335 (0.1 mg/kg, *sc*) only insignificantly attenuated it. On the other hand the NMDA complex agonist N-methyl-D-aspartic acid (NMDA) (75 mg/kg, *ip)* potently ($p < 0.001$) reduced the anti-immobility time of MTEP (0.3 mg/kg), but the AMPA receptor antagonist NBQX (10 mg/kg, *ip*) was inactive. Our results suggest that the antidepressant-like activity of MTEP in the forced swim test in mice involves apart from glutamategic neurotransmission also interaction with serotonergic system.

Does receptor activation initiate the NFκB pathway in hippocampal neurones?

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Over-expression of genes is implicated as a cause of several CNS disease states. Nuclear factor κB (NFκB) is a transcription factor which plays a pivotal role in the regulation of gene expression in a wide range of biological processes in mammals including immunological and inflammatory responses and the regulation of cell proliferation and apoptosis. It has also been shown to play a role in neurodegenerative and neuroprotective processes. However, the precise mechanisms by which components of the NFκB pathway are involved in mediating these processes are not yet clear. This aim of this study was to investigate the modulation of the NFκB pathway by receptor activation in hippocampal neurones utlising immunohistochemical and immunoblotting techniques. Immunohistochemical analysis showed that activation of group I metabotropic glutamate receptors (group I mGlus) and non-NMDA glutamate receptors cause an increase in p-p65 and a decrease in IκBα levels with no change in total p65. An increase in p-p65 was also showed after treatment with the Toll-like receptor 3 (TLR3) activator, poly I:C. Furthermore, age-dependent effects were observed in primary neuronal cultures treated with poly I:C and the proteinase-activated receptor activating peptide, SLIGRL-NH2. Immunoblotting analysis confirmed the immunohistochemical data. These data indicate that activation of group I-mGlus, ionotropic non-NMDA receptors, TLR3 and protease-activated receptor-2 (PAR-2) leads to NFκB pathway activation. As the aforementioned receptor types are proposed to play a key role in many neurodegenerative processes, the results obtained in this study may help to understand the precise mechanisms underlying many neurodegenerative diseases including Alzheimer's disease (AD) or Parkinson's disease (PD).

Effect of metyrapone on the fluoxetine-induced change in extracellular dopamine, serotonin and their metabolites in rat prefrontal cortex

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The currently used antidepressant drugs (ADs) show therapeutic efficacy in a monotherapy in ca 60–70% of the depressive patients. Therefore, to improve the therapy, a combination of ADs belonging to different pharmacological groups, or a combination of an AD and a substance that can enhance its effect is used in the clinic. Among the agents that are expected to potentiate the efficacy of ADs are inhibitors of glucocorticoid synthesis, in particular metyrapone (MET). This compound (an inhibitor of the enzyme 11-b-hydroxylase) is effective in adjunctive therapy when it is used in combination with other AD in both treatment-resistant depression and animal models. To understand the mechanism of the clinical efficacy of a combination of an AD and metyrapone in treatment-resistant depression, the present study was aimed at determining the influence of fluoxetine (FLU; a selective serotonin reuptake inhibitor) and MET, given separately or jointly, on the extracellular level of dopamine (DA), serotonin (5-HT) and their metabolites in rat prefrontal cortex of freely moving rats using microdialysis analysed by high performance liquid chromatography (HPLC) with electrochemical detection. FLU (10 mg/kg), given alone, significantly increased DA and 5-HT concentrations in the rat prefrontal cortex. MET (100 mg/kg) per se did not change the level of monoamines. A combination of FLU and MET produced the same change in the efflux of both DA and 5-HT as did FLU alone. However, the latter combination (FLU and MET) produced significantly bigger increases in the levels of extracellullar DA metabolites (3,4-dihydroxyphenylacetic acid, homovanilic acid) and a 5-HT metabolite (5-hydroxyindoloacetic acid) than did FLU alone. The above finding suggest that – among other mechanism – increases in the levels of extracellullar DA and 5-HT metabolites may play a role in the enhancement of FLU efficacy by MET, and may be of crucial importance to the pharmacotherapy of drug-resistant depression.

Potentiation of the antidepressant-like effect of mirtazapine by low doses of risperidone in the forced swimming test in mice

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Major depression occurs in up to 10% of the population, and all the currently used antidepressant drugs (ADs) are effective, in a monotherapy, in ca. 60–70% of patients. Among the agents that are presently expected to potentiate the efficacy of ADs there are atypical antipsychotics (e.g. olanzapine, risperidone). The latter drugs produce minimal extrapyramidal side effects, and have also been found to be effective and tolerable in some patients with treatment-resistant depression. It is proposed that in lower doses, risperidone mainly acts through blocking the 5-HT_{2A} serotonin receptors and in higher doses it blocks D_2 dopamine receptors *in vivo*. To understand the mechanism of the clinical efficacy of an AD and an atypical an-

typsychotic (a combination therapy) in treatment-resistant depression, the present study was aimed at examining the effect of another AD, mirtazapine (MIR, which enhances noradrenaline and serotonin neurotransmission *via* antagonistic action at central α_2-adrenergic receptors), and risperidone, given separately or jointly, on immobility time in the forced swimming test (an animal model of depression) in male C57BL/6J mice. Fluoxetine (FLU) was used as a reference drug. The obtained results showed that MIR and FLU (5 and 10 mg/kg) or risperidone in low doses (0.05 and 0.1 mg/kg) given alone did not change the immobility time of mice in the forced swimming test. Joint administration of MIR (5 and 10 mg/kg) or FLU (10 mg/kg) and risperidone (0.1 mg/kg) exhibited antidepressant-like activity in the forced swimming test. WAY 100636 (a 5-HT_{1A} receptor antagonist) inhibited, while yohimbine (an α_2-adrenergic receptor antagonist) potentiated the antidepressant-like effect induced by co-administration of MIR and risperidone. The obtained results indicate that risperidone applied in a low dose enhances the antidepressant-like activity of MIR and FLU, and that, among other mechanisms, 5-HT_{1A}-, and α_2-adrenergic receptors may play some role in this effect.

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Immunoreactivity of peritoneal and pleural cells in relation to behavioral responsiveness to stress in rats

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The chronic mild stress (CMS) procedure was developed as an animal model of depression, in which a decreased reactivity to rewards is the major behavioral endpoint [Willner, Neuropsychobiology, 2005]. However, some literature data reported behavioral response to CMS of opposite direction, i.e. increased consumption of sweet solutions [Murison & Hansen, Integr Physiol Behav Sci, 2001]. Depression is also related to disturbances in the immune system, especially to the enormous activity of macrophages. Stress, which can precipitate depression, can also promote inflammatory response [Raison et al., Trends Immunol, 2006]. Thus, the aim of the present study was to explore, whether activity of macrophages is related to the behavioral response to stress in the CMS model of depression. The rats were subjected to the CMS procedure [Papp et al., Neuropsychopharmacology, 2003] and classified on the basis of consumption of sweet solution to three groups as: non-responsive (no changes), responding by diminished intake and responding by increased drinking of sweetened water. Non-stressed animals served as the control group. After decapitation, peritoneal and pleural cells were eluted and some parameters of immunoreactivity were assessed colorimetrically. Viability of the cells was assessed with flow cytometry. We found that peritoneal macrophages showed lower ability to the NO synthesis and lower arginase activity than pleural ones. They also differed in their responsiveness to stimulation with lipopolysaccharide (LPS). Pleural macrophages obtained from non-responders revealed increased synthesis of NO. Peritoneal macrophages obtained from rats responding by diminished uptake of sweetened water showed increased spontaneous arginase activity. Viability of pleural cells was higher in responding rats (regardless of the outcome) whereas viability of peritoneal cells was decreased in all stressed groups. Our results suggest that stress can alter reactivity of macrophages in a pattern to some extent related to the behavioral outcome.

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Memory effect of veratridine in rats

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Veratridine is a natural alcaloid isolated from seeds *Schoenocaulon officinale – Liliaceae*. The mechanism of veratridine action is connected with opening voltage-dependent sodium channels. It causes generation and persistence of neuronal action potential. Disturbances of action of sodium channels are a cause of formation pathological discharges in central nervous system manifested as epilepsy. Moreover pathology of sodium channels favours to dangerous cardiac episodes, e.g. ventricle fibrillation.The aim of this study was to check theory that enlargement activity of neurons through changing action of sodium channels can improve memory consolidation. Experimens were performed on female Wistar rats. 7 days before planned investigations the rats were underwent procedure of implantation of polythene cannulas into the right lateral ventricle of the brain (*icv*) under xylazine and ketamine anaesthesia using technique elaborated in our laboratory. On the day of experiment veratridne was injected to unanaesthetized rats through implated cannulas directly into the right lateral brain ventricle at doses 0.05–65 nmol. Memory effect was determined by a water maze technique of Plech et al. and by an active avoidance test. It was found that veratridine induced biphasic, dose-dependent effect in a water maze test. Lower doses of veratridine improved while higher inhibited rats memory. The results obtained in active avoidance test showed only minimal impairment on memory in rats. There are necessary successive experiment to estimate the role of sodium channels in memory processes.

Effect of alloferon 1 on central nervous system in rats

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Alloferon 1 is an insect-derived peptide with potent antimicrobial and antitumor activity. It was isolated isolated from blood of an experimentally infected insect, the blow fly Callifora vicina. Synthetic alloferon 1 reveals a capacity to stimulate activity of NK cells and synthesis IFN in animal and human models. Moreover it was demonstrated antiviral and antitumoral activity of alloferon 1 in mice. There are no data on influence of alloferon 1 on central nervous system. The aim of present study is to determine an effect of alloferon 1 on rats central nervous system by some behavioral tests: open field test, hole test, score of rats irritability, and determination of memory cosolidation in the water maze test. Moreover a probable antinociceptive effect alloferon 1 in rats it was determined by a tail immersion test and hot plate test. Experiments were performed on female Wistar rats. 7 days before experiments rats were anaesthetized with ketamine and xylazine and polyethylene cannulas were implanted into the right lateral brain ventricle (*icv*). On the day of experiment alloferon 1 dissolved in a volume of 5 μl of saline was injected directly *icv* through implanted cannulas at doses of 5–100 nmol. It was found that alloferon 1 had slight effect on loco-

motor and exploratory activity, induced some decrease of rat irritability and a weak impairment rats memory (only at the low dose of 5 nmol). On the other hand thr higher dose of this peptide exerts significant antinociceptive effect. Obtained results indicate that alloferon 1 do not exert any evidently toxic effect on central nervoust system in rats. Therefore alloferon 1 may be good new drug with antitumor and antinociceptive activity.

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Effect of sigma (σ) receptor ligands on the dizocilpine-induced prepulse inhibition (PPI) deficit in mice

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The sigma (σ) receptors were first defined as a subtype of opioid receptors but later they were found to be a distinct pharmacological entity. The σ receptors are classified into σ1 and σ2 subtypes of which the first was cloned from rodent and human tissues while the second has not yet been fully characterized. Preclinical and clinical data have indicated that σ receptors can be involved in neuropsychiatric disorders, including schizophrenia, and that σ receptor ligands (antagonists) alleviate negative symptoms of schizophrenia. The prepulse inhibition (PPI), a phenomenon in which a weak prepulse stimulus attenuates the response to a subsequent startling stimulus is utilized as a measure of efficacy of sensorimotor gating and is significantly reduced in schizophrenic patients. Noncompetitive NMDA receptor antagonists, such as phencyclidine (PCP) or dizocilpine, have been found to disrupt PPI in animals. On the other hand, antipsychotic drugs are able to reverse dizocilpine-induced deficits in PPI. The aim of this study was to find out whether BD-1047 and SM-21, the σ1 and σ2 receptor antagonists, respectively, are able to modify the dizocilpine-induced PPI impairment in C57BL/6J male mice. The results indicated that BD-1047 (1 mg/kg, *ip*) partly attenuated the PPI deficit induced by dizocilpine, while SM-21 (3 mg/kg, *ip*) had no effect. None of the compounds modified the dizocilpine-induced increase in startle amplitude. As shown previously, BD-1047 induced some effect in behavioral models predictive of antipsychotic activity (antagonism to apomorphine-induced climbing or stereotypy, PCP-induced head weaving). Interestingly, SM-21 alone, like dizocilpine, provoked the PPI disruption in mice. Risperidone (1 mg/kg, *ip*), an atypical antipsychotic drug used for comparison, partly attenuated dizocilpine-induced PPI impairment. The results support the idea that σ1 receptor antagonism may be one of the important mechanisms of antipsychotic activity. The question if pro-mnesic effect of SM-21 is a characteristic feature of σ2 receptor antagonists requires further studies.

Ontogenetic exposure of rats to pre- and post-natal manganese enhances behavioral impairments produced by perinatal 6-hydroxydopamine

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Rats lesioned shortly after birth with 6-hydroxydopamine (6-OHDA; 134 ug *icv*) represent a near-ideal model of severe Parkinson's disease because of the near-total destruction of nigrostriatal dopaminergic fibres. The element manganese, an essential co-factor for many enzymatic reactions, itself in toxic amount, replicates some clinical features similar to those of Parkinson's disease. The aim of this study was to examine the effect of neonatal manganese exposure on 6-OHDA modeling of Parkinson's disease in rats. Manganese ($MnCl_2 \cdot H_2O$), 10,000 ppm was included in the drinking water of pregnant Wistar rats from the time of conception until the 21st day after delivery, the age when neonatal rats were weaned. Control rats consumed tap water. Other groups of neonatal rat pups, on the 3rd day after birth, were pretreated with desipramine (20 mg/kg, *ip* 1 h) prior to bilateral *icv* administration of 6-OHDA (30, 60 or 137 ug) or its vehicle saline-ascorbic (0.1%) (control). At 2-months after birth, in rats lesioned with 30, 60 or 134 µg 6-OHDA, endogenous striatal dopamine (DA) content was reduced, respectively by 66%, 92% and 98% (HPLC/ED), while co-exposure of these groups to perinatal manganese did not magnify the DA depletion. However, there was prominent enhancement of DA D1 agonist (i.e., SKF 38393) -induced oral activity in the group of rats exposed perinatally to manganese and also treated neonatally with the 30 mg/kg dose of 6-OHDA. The 30 mg/kg 6-OHDA group, demonstrating cataleptogenic responses to SCH 23390 (0.5 mg/kg) and haloperidol (0.5 mg/kg, *ip*), developed resistance if co-exposed to perinatal manganese. In the group exposed to manganese and lesioned with the 60 mg/kg dose of 6-OHDA, there was a reduction in D2 agonist (i.e., quinpirole, 0.1 mg/kg) -induced yawning. The series of findings demonstrate that ontogenetic exposure to manganese results in an enhancement of behavioral toxicity to a moderate dose of 6-OHDA, despite the fact that there is no enhanced depletion of striatal DA depletion by the manganese treatment.

Effect of acamprosate on ghrelin blood level in alcohol Warsaw High Preferring and Warsaw Low Preferring rats

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The findings suggest that neuroendocrinological mechanisms are highly involved in the neurobiology of alcohol craving. Recent evidence has suggested that ghrelin, insulin and leptin and the volume-regulating hormones could play a role in the alcohol-seeking behavior [Addolorato et al., Drug Alcohol Rev, 2009]. Acamprosate (AC) has been found beneficial for the treatment of alcoholism, because it reduces relapse in abstinent alcohol-dependent patients [Spanagel and Kiefer, Trends Pharmacol Sci, 2008], but many aspects of its pharmacological profile are still under investigation [Chau et al., Alcohol Clin

Exp Res, 2010]. The aim of this study was to assess the effect of AC on ghrelin plasma level in the model of alcoholism. The experiments were performed on Warsaw High Preferring (WHP) and Warsaw Low Preferring (WLP) of male rats received 10% ethanol using voluntary intake procedure for 4 weeks. Next, after 2-week withdrawal period the animals were treated with AC (500.0 mg/kg, *po*). for 21 consecutive days and both total and acylated ghrelin (active form) levels in plasma of rats were measured using ELISA method. It was found out that WHP rats differ from their counterparts WLP in alcohol intake. With respect to drinking pattern in the investigated rats, it was noticed that there were no statistically significant differences in daily total fluid intake or body mass after the experiment. Both total and active ghrelin levels were significantly decreased in WHP rats when compared with WLP animals. AC treatment lowered alcohol intake in WHP animals, but not that of WLP rats. It corresponded with significant increasing of both active and total ghrelin levels in AC-treated WHP rats, whereas AC lowered the levels of ghrelin in WLP animals. In conclusion, it was found out that the increased ghrelin level after AC administration in WHP rats is negatively coupled with alcohol intake.

Involvement of nitric oxide (NO) in the development of sensitization to diazepam withdrawal signs in mice

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Benzodiazepines are widely used as hypnotics, anxiolytics and anticonvulsant. Their effects are mediated by the γ-aminobutyric acid A ($GABA_A$) receptor complex where they bind to distinct modulatory sites and facilitate the actions of GABA. Repeated administration of benzodiazepines can alter $GABA_A$ receptors, which contributes to the development of tolerance and dependence and often limits their clinical use. NO is produced from L-arginine by a reaction catalyzed by NO synthase in response to activation of excitatory amino acid receptors. It acts as an endogenous activator of guanylate cyclase and thereby increases the level of an intracellular second messenger, cGMP. NO appears to be a novel neuronal messenger involved in a number of physiological and pathophysiological processes. Recent studies indicate that NO may play a role in tolerance, dependence and sensitization to the addictive drugs such as opioids, ethanol, psychostimulants and nicotine. The present studies were undertaken to determine the influence of L-arginine : NO : cGMP pathway modulators (non-selective NO synthase inhibitor: NG-nitro-L-arginine methyl ester (L-NAME), selective inhibitor of neuronal NO synthase: 7-nitroindazole, substrate for NO formation: L-arginine and guanylate cyclase inhibitor: methylene blue) in the development of sensitization to diazepam withdrawal signs in mice. In order to show the sensitization to benzodiazepine withdrawal signs the animals were divided into groups: the animals continuously (for 21 days) treated with diazepam (15 mg/kg/day, *sc*) and the animals receiving diazepam during three 7-day periods interspersed with 3 day diazepam-free period in which the animals were treated vehicle injections. The modulators of nitricoxidergic system were administrated in sporadic diazepam treated mice during the diazepam-free periods (three, daily injections in each of the periods). In all animals, the intensity of diazepam withdrawal signs, observed as the increase in seizure activity (in pentylenetetrazole (PTZ)-induced seizures model) was assessed 48 h after the last injection of diazepam or vehicle. The animals, after concomitant administration of subthreshold dose of PTZ (55 mg/kg, *sc*) with flumazenil (5 mg/kg, *ip*), were placed in glass cylinders and were observed for 1 h. The present studies showed that administration of L-NAME (100, 200 mg/kg, *ip*), 7-nitroindazole (20, 40 mg/kg, *ip*) and methylene blue (5, 10 mg/kg, *ip*) during two diazepam drug-free peri-

ods in sensitized mice, significantly attenuated their seizures activity. L-arginine (250, 500 mg/kg, *ip*) had no effect on diazepam withdrawal-induced sensitization in mice. These results support the hypothesis that nitric oxide is involved, at least partly, in the mechanisms of sensitization to withdrawal signs precipitated after sporadic treatment with diazepam.

Cognitive impairment caused by stress is countered by the long-term administration of Ω-3 polyunsaturated fatty acids

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Cod liver oil (CLO) is a rich source of omega-3 fatty acids (FAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) – the main active agents in fish oil. In this study we tested a hypothesis that DHA and EPA alleviate negative impact of prolonged restraint stress on cognititive functions. Specifically, we attempted to characterize in rats the preventive action of long-lasting treatment with CLO (equivalent to dose 300 mg/kg DHA and 225 mg/kg EPA, *po* for 21 days) against impairments caused by chronic restraint stress on recall, as tested in a passive avoidance task and on the spatial reference and working memory as tested in Barnes maze (BM). CLO significantly improved hippocampus dependent spatial memory and recall in comparison with control ($p < 0.01$) and alleviated some other negative effects of stress on cognitive functioning. In conclusion, the present study demonstrated that prolonged treatment with a standardized, high-concentration DHA-containing, and EPA-containing fish oil reduced stress-induced amnesia as measured in the passive avoidance task as well as alleviated spatial reference and working memory impairments evoked by chronic stress. The present findings not only corroborate the sparse literature concerning the behavioral effects of DHA but also demonstrate for the first time that the use of a CLO facilitates functional recovery after stress including functioning of the brain.

Neuroprotective effects of adenosine A2A antagonists in rats chronically infused with MPP+ into the cerebral ventricle through Alzet osmotic minipumps

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by a loss of dopaminergic neurons in the substantia nigra (SN), and a drop in dopamine (DA) level in the striatum (CP). The current pharmacotherapy is based on the "DA replacement" strategy, however, it does not halt progression

of the degenerative process. Adenosine A2A receptor (A2AR) antagonists stimulate motor activity and provide symptomatic relief in animal models of PD and in parkinsonian patients. Experimental evidence has raised the possibility that these compounds might possess also neuroprotective properties in PD. The aim of the study was to search for the protective effects of the A2AR antagonists: KW6002 (3 mg/kg, *po*), and MSX-3 (1 mg/kg, *ip*), in the chronic model of early stages of PD in which rats received 28-day constant infusion of MPP+ iodide (0.284 mg/kg/day) into the cerebral ventricle using an ALZET osmotic minipump. The A2AR antagonist partially reversed the MPP+ -induced depletion of DA and its metabolites (by 37–50%) in the CP, ipsilateral to the infusion side and the decrease in the number and density of TH-ir neurons in the SN estimated stereologically. However, KW6002 per se markedly increased the level of DA and its metabolites in the CP (by 50–114%) which might be due to its known inhibitory effect on the MAO-B, enzyme involved in the DA metabolism. KW6002 depressed also the small increase in A2AR mRNA expression found in the CP and reversed a reduction in the BDNF mRNA in the CA3 region of the hippocampus. The reversal of a moderate neurodegeneration in the nigrostriatal system and neurochemical changes induced by chronic MPP+ infusion support the role of A2AR antagonists as neuroprotective compounds which may slow down the progression of PD.

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Antipsychotic properties of 1-methyl-1,2,3,4-tetrahydroisoquinoline demonstrated on amphetamine-induced behavioral and neurochemical changes in rat: *in vivo* and *ex vivo* studies

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1-Methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) exhibits neuroprotective, antiaddictive and MAO-inhibiting properties [Antkiewicz-Michaluk et al., J Neurochem, 2006; Wąsik et al., J Physiol Pharmacol, 2007] and may be endogenous regulator of dopaminergic activity. We demonstrated that 3-methoxy-tyramine (3-MT), which behaved as antagonist of the catecholaminergic system [Antkiewicz-Michaluk et al., Eur J Pharmacol, 2008] may play an important role in 1MeTIQ mechanism of action. In rodents, amphetamine is often used to evoke schizophrenia-like behavioral abnormalities. The aim of present study was investigate potential antipsychotic properties of 1MeTIQ. We tested the influence of 1MeTIQ on amphetamine-induced hyperactivity and changes in dopamine metabolism in rat brain. We also analyzed *in vivo* the effect of 1MeTIQ on release of dopamine produced by amphetamine in rat striatum. The behavioral tests have shown that 1MeTIQ significantly antagonized amphetamine-induced hyperactivity in rats. On the other hand in biochemical experiments 1MeTIQ, like to MAO inhibitors, significantly intensified amphetamine-induced attenuation of dopamine metabolism in striatum and nucleus accumbens. *In vivo* microdialysis studies showed that amphetamine produced a significant increase (by about 700 %, $p < 0.01$) of an extraneuronal concentration of DA whereas the level of its metabolites, DOPAC and HVA were decreased. 1MeTIQ alone, produced an elevation of DA release (by about 200%) with simultaneously a strong increased of 3-MT and decreased of DOPAC and HVA. Combined treatment of 1MeTIQ with amphetamine has shown an increase (by approx. 2500 %, $p < 0.01$) of extraneuronal DA concentration. In the same time the concentration of 3-MT was powerfully elevated (approximately 6,000%, $p < 0.01$). 1MeTIQ strongly affected the mechanism of action of amphetamine. A huge rise of 3-MT concentration in extracellular area in the case of com-

bined treatment of 1MeTIQ with amphetamine may be responsible for 1MeTIQ-produced behavioral antagonism to amphetamine-hyperactivity in rats. If the hyperlocomotion elicited by acutely administered amphetamine is a valid model of at least in some aspects of schizophrenia, these results indicate that 1MeTIQ exhibits antipsychotic-like efficacy, and may be useful in clinical practice as a psychosis-attenuating drug in schizophrenic patients.

Siglec-F receptor and neuroprotection in mouse central nervous system

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Neuroprotective function of Siglec-F receptor in mouse central nervous system.

Sialic acid-binding immunoglobulin superfamily lectins (Siglec) are members of immunoglobulin superfamily that recognize sialic acid residues of glycoproteins. Siglec-F is a CD33-related Siglec that binds to 2.3-, 2.6- and weakly to 2.8-linked sialid acid. We analysed distribution and function of Siglec-F receptor in neurodegenerative processes. We observed Siglec-F gene transcription and Siglec-F protein expression in C57BL6 mice immunized for Experimental Autoimmune Encephalomyelitis (EAE). Real-Time-PCR and Western Blot analysis of EAE brain and spinal cord tissues showed increased microglial activation and Siglec-F expression. Siglec-F deficient mice (–/–) presented an altered development of EAE. Proinflammatory stimulation of cultured mouse microglia increased Siglec-F gene transcription. Coculture of microglia and neurons demonstrated neuroprotective function of sialic acid receptors. Neuroprotective effects of Siglec-F were dependent on sialic acid residues on neurons. Absence of sialic acid changed microglial-neuronal interactions and caused dramatic reduction of neurites and neuronal cell bodies numbers. These data demonstrate that activation of innate immune receptors, like Siglec-F, can modulate mouse microglia activity and may represent a new therapy in neurodegeneration.

The influence of D-cycloserine and midazolam on the release of glutamate and GABA in the basolateral amygdala of low and high anxiety rats during extinction of a conditioned fear

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In this study, we investigated how D-cycloserine and midazolam regulate fear extinction and reactivity of brain neurotransmitter systems to fear-evoking context in rats with varying intensities of a fear response.

The rats were divided according to their behavioral responses in the conditioned fear test (CFT): HR – (high responders, freezing time longer than the mean ± SEM) and LR (low responders, freezing time shorter than the mean ± SEM). On the 8[th] day after CFT, the animals were exposed again to the aversive context (extinction sessions). To assess the glutamate and GABA release in the basolateral amygdala (BLA) we used the method of microdialysis *in vivo*. The results showed that D-cycloserine (15 mg/kg, *ip*, given 30 min before the extinction session) significantly enhanced an inhibition of an aversive context-induced freezing response observed during extinction session in HR and LR rats, while midazolam (0.75 mg/kg, *ip*, 30 min before the extinction) accelerated the attenuation of fear responses only in HR rats. The less anxious behavior of LR animals given saline was accompanied by elevated levels of glutamate and GABA in the BLA, in comparison with HR rats, and a stronger elevation of GABA in response to contextual fear. In more anxious HR animals, the pretreatment of rats with D-cycloserine and midazolam significantly increased the local concentration of GABA and inhibited the expression of contextual fear. These findings suggest that animals more vulnerable to stress have innate deficits in brain systems that control the activity of the amygdala mediating the central effect of stress and may help to better understand the mechanism of individual differences in the anxiolytic drugs, found among the patients with anxiety disorder.

Hypocretin type 1 receptor (Hcrtr-1), but not Hcrtr-2, stimulates cAMP production in primary glial cell cultures from rat cerebral cortex

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Hypocretin-1 and hypocretin-2 (also called orexin A and orexin B, respectively) are recently discovered neuropeptides synthesized by a specific population of neurons in the lateral hypothalamus. Hypocretins have been implicated in a variety of behaviors, e.g. arousal and sleep, food-seeking and feeding, reaction to stress. Disturbances in the central hypocretin neurotransmission are believed to underlie narcolepsy. It has also been postulated that hypocretins play an important role in the regulation of HPA axis, energy homeostasis, acquisition and learning of reward system-stimulating signals. The physiological effects of hypocretins are mediated *via* two specific, membrane-bound, G protein-coupled receptors: Hcrtr-1 (OX1R) and Hrctr-2 (OX2R). Hcrtr-2 is nonselective for the two neuropeptides, whereas Hcrtr-1 exhibits substantially higher sensitivity for hypocretin-1. Signal transduction systems linked to activation of hypocretin receptors are yet poorly understood. In this study we examined effects of stimulation of hypocretin receptors on cAMP formation in primary glial cell cultures from rat cerebral cortex (CCx). Hypocretin-1 (0.001–1 μM) increased, in a concentration-dependent manner, basal cAMP production in glial cell cultures, with an EC50 value of 0.7 μM. The peptide markedly potentiated the stimulatory action of forskolin (a direct activator of adenylyl cyclase) on cyclic AMP formation. [Ala11-D-Leu15ypocretin-2 (a selective agonist of Hcrtr-2 receptors) did not modify basal and forskolin-stimulated cAMP production in glial cell cultures from rat CCx. The studied effects of hypocretin-1 were blocked by SB 408124, a selective antagonist of Hcrtr-1 receptors, and not affected by TCS OX2 29, a selective antagonist of Hcrtr-2 receptors. It is suggested that in glial cell cultures from rat CCx activation of Hcrtr-1 receptors stimulate cAMP production.

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Effects of self-administered cocaine in rats on accumbal and pallidal dopamine, glutamate and GABA levels

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Cocaine abuse and addiction is the serious medical and social worldwide problem [Xi and Gardner, Curr Drug Abuse Rev, 2008]. Current methods of treatment of cocaine addiction are not effective, probably due to the fact that the mechanisms underlying drug abuse are not yet fully understood.

The present study aimed to analyze the concentration of dopamine (DA), glutamate (Glu) and γ-aminobutyric acid (GABA) during intravenous cocaine self-administration in the nucleus accumbens and ventral pallidum in male Wistar rats. The use of "yoked" procedure in the self-administration model enabled to separate the pharmacological effects of cocaine from the effects evoked by the motivational and cognitive processes associated with active cocaine administration. Thus, neurochemical changes with using microdialysis were tested in rats which received active cocaine and in animals that received passively either cocaine or its vehicle. The levels of DA, Glu and GABA were assayed with the use of high performance liquid chromatography. In rats actively administering cocaine (0.5 mg/kg/infusion; 2-h session over 6 days/week), an increase in DA synaptic concentration concurrent with the decreases in Glu and GABA levels were observed in the nucleus accumbens. In the same animal group, a reduction in the Glu level with no significant changes in GABA concentration were noted in the ventral pallidum. In animals receiving cocaine passively, an increase in accumbal DA was seen, however, this effect was less evident and short-lasting when compared to rats actively taking cocaine. Our study indicates that cocaine (either active or passive injections) increases accumbal DA level, while decreases in Glu concentration – being observed only in animals administering cocaine actively – are associated with the motivational effects of cocaine.

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Optimization of the method for determination of carbamazepine in plasma and brain tissue

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Carbamazepine (CBZ) is the basic substance used in the treatment of partial and tonoclonic epileptic seizures, and in the treatment of neuralgic pain of trigeminal and glossopharyngeal nerves. The purpose of the present study was optimization of method for detection of CBZ in plasma of human and 10 different animal species, as well as in homogenates of pig brains. An essential element of the study was selection of phase liquid liquid extraction (LLE) and choice of suitable modifying agent to improve the release of CBZ from the plasma protein-bound fraction, as well as from the brain tissue. The analysis was performed on a liquid chromatograph Waters Alliance 2695, with a PDA detector (λ = 210 nm), using column Grace 4.6/150 mm, 3 μm. The metod detection limit was 100 ng/ml of plasma and 150 ng/g of brain

Extractant (LLE)	pH of samples	Recovery $_{LLE}$ (%)		Modifier (1) + ethyl acetate (2)	Recovery $_{1+2}$ (%)	
		plasma (0.5 ml)	brain* (0.2 g)		plasma (0.5 ml)	brain* (0.2 g)
1,2-dichloroethane	< 7.0	0.8	33.8			
Ethyl acetate		61.5	35.1	0.5 ml ACN : NH_4 (96:4; v/v)	66.3	–
n-Butyl acetate		77.6	–	0.75 ml ACN : NH_4 (96:4; v/v)	79.1	–
Diethyl ether		29.3	–	1.0 ml ACN : NH_4 (96:4; v/v)	75.9	–
Methyl *tert*-butyl ether		37.6	–			
Hexane		–	–	150 µl 10% HCOOH	39.3	–
1,2-dichloroethane	> 7.0	4.4	–	150 µl 6% NH_3	68.7	73.9
Ethyl acetate		62.4	90.1			
n-Butyl acetate		79.6	62.7	150 µl 0.8% SDS	52.2	–
Diethyl ether		31.6	–	300 µl 0.8% SDS	–	–
Methyl *tert*-butyl ether		37.4	–	600 µl 0.8% SDS	–	–
Hexane		–	2.7			

– low recovery or poor chromatography; * pig brain homogenates.

tissue. The results obtained indicate high sensitivity of the method as compared to those found in the literature. The most important results are presented in the table (see above).

The method described had specificity and selectivity for plasma collected from all species as well as for the pig brain tissue. The limit of quantitation (LOQ) for plasma was 100 ng/ml and for brain was 150 ng/g tissue. Linearity of the method was confirmed for ten points in the range of concentration 100 ng/ml – 10 µg/ml for plasma, and 150 ng/g – 10 µg/g for the brain tissue.

Model-independent method of the analysis of distribution of 1,4-benzodiazepine derivatives possessing psychotropic activity after a single administration in the mice

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The kinetic scheme of distribution of a structural analogue of phenazepam [14C]-7-brom-5-phenyl-1,2-dihydro-3H-1,4-benzdiazepine-2-on (I) and its main [14C]-metabolites in the mice was presented. It was shown, that compound I is metabolized to active metabolite 3-hydroxyphenazepam. Compound (I) and its active metabolite were rapidly distributed in the body of mice. A rapid phase of distribution of [14C]-I has been noted that faded already during the first hour following drug administration. The highest total radioactivity was observed in liver and kidneys of mice. The lowest radioactive counts have been found in plasma. It was shown that a fatty tissue can function as the peripheral compartment of the kinetic scheme of the drug distribution. One of basic parameters characterizing the processes of drug distribution between blood, as a central compartment of kinetic schemes of distribution and organs or tissues as peripheral com-

partment of organism it is been equilibrium constant An integral model-independent method for estimation of equilibrium tissue-to-plasma partition ratios and rate of the reverse process from tissues in blood has been proposed. A comparative analysis of existing pharmacokinetic methods for estimation these parameters was carried. The advantage of the proposed method over that of the comparison method is that it is correct for different schemes administration of drug and does not depend on the structure of the kinetic scheme and, most importantly, can be used for analysis of incomplete kinetic curves. Thus, it can be used in the case of the xenobiotic mass transfer in conditions of a poorly defined shape of the kinetic curve, which lacks some phase (absorption, distribution or elimination) during the interval of observation. The applicability of this formal approach has been practically validated on the example of the distribution of compound I in mice after single of its administration.