Plenary lectures

Monoamine transporter inhibitors and norepinephrine inhibit dopamine-dependent iron neurotoxicity

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Iron overload in the brain has been associated with several neuropathologies such as (i) Parkinson´s disease since a significant increase in iron content has been observed in substantia nigra of both post-mortem parkinsonian brain and in the living patients using imaging techniques; (ii) the hereditary deficiency of ceruloplasmin in aceruloplasminemia, which is associated to both basal ganglia degeneration and iron accumulation in the brain. In this regard, ferroxidase activity of ceruloplasmin was reportedly decreased by 40% in untreated patients with Parkinson´s disease.

The finding that inhibitors of monoamine transporters strongly decrease the cell death in RCSN-3 cells treated with Fe-dopamine complex plus dicoumarol suggests a possible therapeutic use of compounds which block monoamine reuptake in preventing iron-dependent toxicity in catecholaminergic neurons in diseases which involve iron accumulation in the brain. RCSN-3 cells express tyrosine hydroxylase and transporter for dopamine, norepinephrine and 5-hydroxytryptamine determined by immunohistochemistry. In RCSN-3 cells, the uptake of 100 μM $^{59}FeCl_3$ alone was 25 ± 4 nmol/min/mg and that complexed with dopamine (Fe(III)-dopamine) increased to 28 ± 8 was nmol/min/mg, which was inhibited by 2 μM nomifensine (43%; $p < 0.05$), 100 μM imipramine (62%, $p < 0.01$), 30 μM reboxetine (71%; $p < 0.01$) and 2 mM dopamine (84%; $p < 0.01$). No toxic effects in RCSN-3 cells were observed when the cells were incubated with 100 μM $FeCl_3$ alone or complexed with dopamine. However, 100 μM Fe(III)-dopamine in the presence of 100 μM dicoumarol, an inhibitor of DT-diaphorase, induced toxicity (44% of dead cells; $p < 0.001$), which was inhibited by 2 μM nomifensine, 30 μM reboxetine and 2 mM norepinephrine. The neuroprotective action of norepinephrine can be explained by (i) its ability to form complexes with Fe^{3+}; (ii) the uptake of Fe-norepinephrine complex through norepinephrine-transporter; and (iii) the Fe-norepinephrine complex is not toxic even when DT-diaphorase is inhibited. These results support the idea to use inhibitors of monoaminergic transporters to prevent Fe-neurotoxicity under iron overload conditions.

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Why drinkers smoke? A pharmacological perspective

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A heavy drinker is likely to be a heavy smoker as well. This happens, despite the indications that such combination can dramatically increase the risk of various cancers. In this presentation, three potential contributory factors to alcohol-nicotine co-abuse are discussed. These include: 1. Potentiation of the rewarding effects, reflected in exaggerated release of dopamine in the nucleus accumbens (NACC) following co-administration of alcohol and nicotine. 2. Potentiation of the analgesic effects when a combination of sub-maximal doses of alcohol and nicotine are given to experimental animals. 3. Counteraction of alcohol-induced toxicity by nicotine in an *in vitro* primary cell culture model.

Following a brief discussion on involvement of the mesolimbic dopaminergic pathway in reward mechanism, data is provided showing that when a low to moderate dose of alcohol administered systemically is combined with a low dose of nicotine administered directly into the ventral tegmental area, an additive release of dopamine in NACC is obtained. Moreover, the effects of alcohol on dopamine release can be blocked by nicotinic antagonists, implying a role for nicotinic receptors in alcohol-induced reward and, hence, therapeutic potential for selective nicotinic antagonists in alcohol addiction.

Data are also presented, indicating that when sub-maximal doses of alcohol and nicotine, i.e. doses that do not induce analgesia on their own, are combined together, an analgesic effect is obtained. This analgesic effect is manifested in both tail flick and hot plate tests, thus implicating both spinal and supraspinal analgesic pathways. Furthermore, the antinociceptive effects of combined alcohol and nicotine are attenuated or completely blocked by naloxone, a non-selective opioid receptor antagonist suggesting a therapeutic potential for selective opioid antagonists in co-morbid condition of drinking and smoking.

Finally, using primary cultures of cortical neuronal cells as well as cerebellar granule cells, it is shown that alcohol-induced toxicity in these cells can be blocked by nicotine pretreatment. The effects of nicotine, in turn, can be blocked by several nicotinic receptor antagonists. Data are provided indicating that apoptotic mechanism, reflected in caspasse-3 activation, is at least partially responsible for the toxicity induced by alcohol. The results, in addition to suggesting a "pharmacodynamic" basis for combining nicotine with alcohol, imply potential application of nicotinic agonists in neurodegenerative disorders underscored by apoptotic mechanisms.

In summary, combined effects of ethanol and nicotine on reward and analgesic pathway as well as counteracting some of the adverse effects of alcohol by nicotine may be contributing factors to the high incidence of smoking in alcoholics. Elucidation of the role of nicotinic and opioid receptors in dual addiction to nicotine and alcohol may lead to novel intervention in smoking and drinking co-morbidity.

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Pathogenesis and treatments of age-related macular degeneration (AMD)

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Age-related macular degeneration (AMD) is one of the most common irreversible causes of severe loss of vision, including legal blindness, in people over 60. Despite intensive basic and clinical research, its pathogenesis remains unclear, and is likely to be of multifactorial character. In addition to age as a primary risk factor, a complex interaction of metabolic, functional, genetic and environmental factors seems to create conditions for chronically developing changes in ocular structures of the macula region (choriocapillaries, Bruch's membrane, retinal pigment epithelium-RPE, photoreceptors) which finally lead to vision deterioration followed by vision loss. Two forms of AMD are classically distinguished: atrophic (dry form) and exudative (wet form). The dry form, also known as geographic atrophy – the most common form (nearly 80 % of all cases of AMD represent this form) - is typically characterized by a progressive atrophy of RPE with a subsequent loss of photoreceptors. The exudative form is linked to choroidal neovascularization directed to the subretinal macular region, with subsequent bleeding and/or fluid leakage, which may result in a sudden loss of central vision; it is the most rapidly progressing form of AMD. Clinical symptoms common for the two types of AMD include the presence of drusen, as well as hypo- and/or hyperpigmentation of the RPE. The pathophysiology of AMD is complex and at least four processes contribute to the disease, i.e. lipofuscinogenesis, drusogenesis, local inflammation and neovascularization (in the case of the wet form).

Lipofuscin represents a complex aggregate lipoprotein materials (that are also oxidized and modified by secondary reactions) accumulating within the lysosomal system during the aging process in many metabolically active postmitotic cells, including RPE. In the case of RPE cells, most of the lipofuscin originates primarily from the incomplete degradation of ingested and then internalized macromolecules of photoreceptor outer segments, including retinoid derivatives. Among the latter, a prominent constituent is a di-retinal conjugate named A2E which behaves as a photoinducible generator of cytotoxic reactive oxygen species. Accumulating evidence suggests that the process of lipofuscinogenesis, with its final damaging impact in especially the aging RPE, may be seriously implicated in pathogenesis of the slowly and imperceptibly developing AMD.

Drusen are extracellular deposits that accumulate between the RPE and Bruch's membrane; they are considered a significant risk factor for the development of the dry and wet forms of AMD. Drusogenesis is a slow process taking place over many months-years - thus, drusen increase in size with age, and recent evidence indicates that they contain molecules derived from multiple sources, including the RPE, choroid, and plasma. Proteomic analysis of drusen revealed the presence within these structures of many proteins that mediate inflammatory processes, including elements of the complement system, suggesting that they may induce chronic localized activation of the complement alternative pathway. Such a suggestion is in line with recent genetic findings showing that in at least 50 percent of AMD-afflicted patients there is a tyrosine›histidine mutation at amino acid 402 (Y402H) of the gene encoding the complement factor H (CFH). As CFH prevents uncontrolled complement activation and inflammation, its mutation is thought to increase inflammation and its consequences, with its negative impact on overlying (and neighboring) RPE cells, particularly those localized to the macular area.

Choroidal neovascularization (CNV) is a major cause of visual loss in AMD. Under normal conditions, endothelial cells lining blood vessels are relatively "silent", and this is because of a functional balance between pro-angiogenic and anti-angiogenic factors. However, under certain conditions, including RPE/photoreceptor degeneration, hypoxia, inflammation (which occur in the macula region in the developing AMD), RPE cells and different "inflammatory" and/or "immune system" cells may produce pro-

angiogenic factors, prominently VEGF (vascular endothelial growth factor), which may lead to functional overactivity of pro-angiogenic signaling with resulting subretinal (choroidal) neovascularization. Since the CNV, with its leaky and bleeding capillary vessels, is the most devastating and dramatic feature of the advanced AMD, it is not surprising that most current therapies (as well as investigational efforts) are directed at this pathology.

Therapy – at present there is no established effective treatment for the dry form of AMD, and as stated above, practically all current medical treatments are designed to combat the existing or to prevent the development of newly formed unwanted blood vessels. Such treatments include photodynamic therapy (PDT) with an intravascular photosensitizer verteporfin, thermal laser photocoagulation, transpupillary thermotherapy (TTT), as well as several new modalities (some of them being still in clinical trials) utilizing agents capable of blocking various steps in the pathway of angiogenesis in the CNV, such as pegaptanib sodium (Macugen), ranibizumab (Lucentis), anecortave acetonide (Retaane), triamcinolon acetonide, or agents being still in preclinical or early clinical trials, e.g. anti-VEGF agents: Sirna-027, VEGF-Trap, or native anti-angiogenic factors, eg. angiostatin, endostatin or pigment endothelium-derived factor (PEDF).

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Symposium

Dedicated to
Prof. dr hab., dr h.c. JERZY VETULANI
on the occasion of 50th anniversary
of his scientific activity

After more than a half of century of psychopharmacology

To the distinguished Professor dr hab., dr h.c. Jerzy Vetulani (his old friend's reflections)

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The first antipsychotic drug chlorpromazine was introduced into the treatment of schizophrenia in 1952. The main problem studied during that time of the origination of the newest field of pharmacology, called psychopharmacology, was to find how neurons communicate between themselves. One of the first pharmacological tools for these studies was reserpine [Bunney and Davis, Arch Gen Psychiatry, 1965]. Between 1965–1970 three substances were called cautiously "putative" neurotransmitters. They were 5-hydroxytryptamine (5-HT) (B. Brodie), noradrenaline (NA) (J. Axelrod) and dopamine (DA) (A. Carlsson). In the late 1970s it was really proven that the mentioned 5-HT and the two catecholamines were the neurotransmitters in the brain. In 1973, O. Hornykiewicz made the essential clinical discovery that the cause of the Parkinson's disease is the DA deficit in the nigrostriatial system.

Before the discovery of these three neurotransmitters, J. Eccles discovered that acetylcholamine was a neurotransmitter in Renshaw cells of the spinal cord (1961).

The next step in deeper understanding of the central nervous system function in normal and pathological conditions was discovery of amino acids. They are very small chemical entities, which are the signals transmitting information very quickly.

The first discovered amino acids were GABA and glycine, which are very potent inhibitory neurotransmitters.

Then excitatory amino acids: L-glutamate and L-aspartate were found. Their concentrations in brain areas are 100 times higher than the level of DA and 5-HT.

Actually, the studies on excitatory amino acids are very active and fruitful.

The next turning point in psychopharmacology was the finding of the brain neuropeptides: enkephalins in 1975, and then endorphins and dynorphins. This discovery changed completely the neurotransmission "dogma" and complicated the actual view on the neurotransmission in the CNS and was a source of several controversies.

A new term "neuromodulators" was coined, which is still poorly defined.

Relatively new neurotransmitters under study: prostanoids, cytokines, tachykinins, nitric oxide are considered by some scientists to be neuromodulators.

The problem of release of co-transmitters is still the hot experimental point.

Synchronously with investigations of neurotransmitters, the vast field of "receptorology" of the mentioned signals was developed.

The above-mentioned effort brought to mentally ill patients a great benefit. During little more than 50 years the antipsychotics, anxiolytics and antidepressants were introduced into medical practice. During the time passing, the more efficacious, active and less toxic drugs are obtained.

After presenting above the history of psychopharmacology in a nutshell, I would like to point out the main contribution of Jerzy Vetulani to psychopharmacology.

Unquestionably his brilliant success was the discovery with F. Sulser of the phenomenon of β-adrenergic receptor down-regulation after chronic treatment with antidepressants, published in Nature, 1975 and Naunyn Schmiedeberg's Arch Pharmacol in 1976.

The next very important steps in his scientific activity are:

– very important finding that clonidine inhibits morphine abstinence syndrome. This discovery is actually applied in the treatment of abstinence syndrome in persons with opioid dependence syndrome (1978);

– the discovery that central α_1-adrenergic receptors and 5-HT_2 receptors may be up-regulated after the treatments with antidepressants (1981);

– the first description of the effects of electroshocks on the central serotoninergic neurons (1981);

– finding that calcium blockers inhibit the rate of morphine dependence and abstinence (1994);

– the proposal of the hypothesis on the adaptative role of calcium ions in the CNS (1995);

– finding of several experimental evidences indicating that endogenous tetrahydroisoquinolines modulate dopamine neuron activity (2001, 2003, 2004);

– the discovery that chronic treatment by antidepressants increases the expression of mRNA encoding adrenergic receptor of 1A type (2002).

Jerzy Vetulani is an excellent popularizer of science. He published a vast number of reviews, notes, popularizing the basic problems of psychopharmacology.

His book "How to Improve Memory" published in 1993, 1995 and 1998 has been a real bestseller.

For several years, he had been the Editor-in-Chief of a prestige journal "The Universe".

The above-described great scientific activity of Jerzy Vetulani generated his scientific school.

I finish this essay by the words of Erich J. Lejeune: "Nothing can restrain the person, who by the honest methods aspires to the noble goal, and strives forward with enthusiasm and trust for fulfillment of his plans".

This quotation characterizes fully Jerzy Vetulani as a personality and scientist.

Selective neurotoxins and neuroscience – approaching the golden anniversary

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In days of yore, axonal transection was the single option for precisely denervating an organ. Nerve toxins, such as heavy metals, were too global in effect. The door to highly selective neurotoxins was opened in the 1960s by Rita Levi-Montalcini, who developed an antiserum to the nerve growth factor (NGF) on which she had worked for more than a decade. This *anti-NGF* antiserum selectively destroyed sympathetic noradrenergic and sensory neurons, both *in vitro* and *in vivo*. When administered to neonatal mice or rats, life-long *chemical sympathectomy* was achieved. A few years after this discovery, Hans Thoenen and J.P. Tranzer, in attempting to find a better chemical analog to darken noradrenergic granules in electron micrographs, serendipitously discovered that one of these analogs, namely 6-hydroxydopamine (6-OHDA) overtly destroyed noradrenergic nerves. The modern era of highly selective neurotoxins was upon us. The properties of a metabolic precursor of 6-OHDA, namely 6-hydroxydopa (6-OHDOPA) was next uncovered by

D.M. Jacobowitz and myself, and the ability of 6-OHDOPA to cross the blood-brain barrier lent yet another dimension to the neurotoxins arena. Selective neurotoxins for other neuronal phenotypes eventually were discovered, thereby expanding the menu for scientific experimentation. The highly selective neurotoxins are used universally and routinely today, to denervate a brain structure, to lesion a brain nucleus, to model a human neurodegenerative disease, and to study degenerative, regenerative, and neuroprotective mechanisms. Selective neurotoxins have had an enormous role in neuroscience discovery. Their history of development, from birth to today's golden years will be outlined, and perhaps an appreciation of their value can be derived by delineating their accomplishments over the past 40 plus years.

Wide perspectives of therapeutic application of an endogenous compound from 1,2,3,4-tetrahydroisoquinoline group

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Tetrahydroisoquinolines (TIQs) such as 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol) and 1-benzyl-1,2,3,4-tetrahydroisoquinoline (1BnTIQ) aroused a considerable interest as molecular species that may be implicated in etiology of Parkinson's disease. They may be formed in the brain enzymatically or non-enzymatically in the Pictet-Spengler reaction from phenylalanine and acetic aldehyde, and they are present also in various foods, such as dairy products, wines and bananas. However, not all TIQs are equally neurotoxic. Recently, the interest of researchers was aroused by an endogenous derivative, 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) which is regarded as a neuroprotectant and was shown to antagonize behavioral syndrome produced by a well- known neurotoxin MPTP [Tasaki et al., Adv Neurol, 1993] The central dopaminergic system plays an important role in motor activity, motivation, learning and reward system. Moreover, the neurotransmitter of this system, dopamine, is a highly reactive compound, and its catabolism connected with oxidation by MAO leads to the formation of DOPAC what is accompanied by generation of free radicals (H_2O_2 and ·OH). It seems that a compound shifting dopamine metabolism from N-oxidation (MAO-dependent pathway) towards the O-methylation (COMT-dependent pathway) may protect the dopamine neurons.

In this lecture I would like to demonstrate the new view on the mechanism of action of 1MeTIQ, an endogenous compound present in the CNS. Our behavioral and neurochemical experiments have shown antidopaminergic [Antkiewicz-Michaluk et al., J Neural Transm, 2000; Antkiewicz-Michaluk et al., J Neurochem, 2001], neuroprotective [Antkiewicz-Michaluk et al., Eur J Pharmacol, 2003], and antiaddictive [Antkiewicz-Michaluk et al., Int J Neuropsychopharmacol, 2004; Antkiewicz-Michaluk et al., Acta Neurobiol Exp, 2005; Wąsik et al., Eur Neuropsychopharmacol, 2005] activity of 1MeTIQ in rodents.

1MeTIQ – dopamine system

1MeTIQ even in the high dose of 100 mg/kg *ip* did not change general behavior of the rats, did not change also the locomotor activity in navie rats but completely antagonized hyperactivity produced by dopamine agonist (eg. apomorphine, amphetamine). 1MeTIQ did not bind to antagonistic conformation of dopamine receptor but displaced ^{3}H-apomorphine from its binding sites with effectiveness comparable to that of dopamine. Moreover, 1MeTIQ administered *in vivo* in high dose (100 mg/kg *ip*) did not change the total metabolism of dopamine in rats but strongly inhibited the dopamine MAO-dependent N-oxidation, and accelerated the COMT-dependent O-methylation. Such

an effect on dopamine catabolism may reduce the generation of free radicals accompanying this process and is interesting in the context of potential clinical application.

The results support the hypothesis that 1MeTIQ may play an important role in regulation of dopaminergic activity in CNS.

1MeTIQ – neuroprotection (*in vitro* and *in vivo* studies)

In vitro studies have shown that 1MeTIQ possesses free radical scavenging properties, as well as inhibits indices of neurotoxicity (caspase-3 activity, lactate dehydrogenase release) induced by glutamate in mouse embryonic primary cell cultures (a preparation resistant to NMDA toxicity). Additionally, in granular cell cultures obtained from 7-day-old rats, 1MeTIQ prevented the glutamate-induced cell death and ^{45}Ca influx. This suggested a specific action of 1MeTIQ on NMDA receptors, which was confirmed by inhibition of [^{3}H]MK-801 binding by 1MeTIQ. We also investigated in *in vivo* studies whether 1MeTIQ exerts a protective action against rotenone-induced changes in dopamine metabolism, mortality and the degeneration of dopaminergic structures. Rotenone was administered systemically as well as structurally into medial forebrain bundle (MFB). We have found that repeated (once daily for seven days) but not single administration of rotenone (12 mg/kg *sc*) causing abnormalities in general behavior of rats, produced considerable mortality, and dramatic increases in the total DA metabolism, which may be ascribed to an increase in the oxidative pathway. These behavioral and biochemical changes were effectively counteracted by administration of 1MeTIQ (50 mg/kg *ip*) before each dose of rotenone. The unilateral injection of rotenone into the left MFB produced neurodegeneration of dopaminergic terminals resulting from a strong decrease (by over 70%, $p < 0.001$) in DA concentration in the ipsilateral striatum, while the DA decline in the ipsilateral SN was much weaker (by 35%, $p < 0.05$). Treatment with 1MeTIQ (50 mg/kg *ip*) before, and three weeks after withdrawal from rotenone given to the left MFB significantly attenuated the fall in DA concentration both in the striatum and SN. Finally, we demonstrated also in an *in vivo* microdialysis experiment that 1MeTIQ (50 mg/kg *ip*) completely prevented kainate-induced (given locally in concentration of 50 μM) release of excitatory amino acids (glutamate and aspartate) from the rat frontal cortex.

Our results indicate that 1MeTIQ may offer a unique and complex mechanism of neuroprotection in various neurodegenerative illnesses of the central nervous system in which antagonism to the glutamatergic system may play a very important role.

1MeTIQ – antiaddictive effect

1MeTIQ strongly influenced the mechanism of action of drugs of abuse: morphine, cocaine and alcohol. 1MeTIQ administered in doses from 12.5 to 50 mg/kg *ip* produced dose-dependently slight analgesia in the "hot-plate" test but strongly potentiated morphine-induced analgesia, as well as prevented the development of morphine tolerance. Expression of morphine-abstinence syndrome (body shakes, head-twitches, and body weight loss) induced by naloxone in morphine-dependent rats was also completely inhibited by pretreatment of 1MeTIQ (50 mg/kg *ip*). Additionally, in the conditioned place preference test (CPP) in mice, the compound inhibited both, the acquisition and expression of CPP without producing either CPP or aversion itself. 1MeTIQ dose-dependently (25, 50 and 75 mg/kg, *ip*) reduced ethanol consumption in rats selected for alcohol preference without changing of total fluid intake. 1MeTIQ (50 mg/kg *ip*) completely inhibited the expression of reinstatement of cocaine self-administration and accompanying neurochemical changes induced by a single priming cocaine dose (10 mg/kg *ip*). The priming cocaine dose inhibited dopamine metabolism in the structures containing nerve endings (frontal cortex, nucleus accumbens, and striatum) but not in the substantia nigra and ventral tegmental area. A behaviorally active dose of 1MeTIQ administered 30 min before a priming dose of cocaine significantly increased the dopamine concentration in the limbic structures, and strongly inhibited dopamine metabolism in the substantia nigra and ventral tegmental area. Cocaine also inhibited noradrenaline and serotonin metabolism, and 1MeTIQ abolished the inhibition in noradrenaline metabolism, while it intensified the inhibition of serotonin metabolism.

The results strongly support the view that 1MeTIQ, an endogenous compound, has considerable potential as a drug for combating substance abuse disease through the attenuation of craving.

Investigations of receptor changes induced by antidepressant drugs in the late 1970s – early 1980s

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The development of receptor binding studies in the late 1970s, together with the discovery of β-adrenoreceptor down-regulation hypothesis of action of antidepressant drugs (AD) prompted us to investigate the effect of action of these drugs on other types of monoaminergic receptors in the brain. Our attention was focused on α_1- and α_2-adrenoceptors as well as on serotonergic receptor in the brain. We discovered that while α_1-adrenoreceptors were up-regulated the α_2-adrenoceptors were down-regulated by antidepressants. Antidepressant-induced changes in the 5-HT_2 receptors were also described. One of the main outcomes of our studies was a discovery that adaptive changes induced by antidepressant drugs concern not only β-adrenoreceptors.

Antidepressant-like actions of nicotinic receptor agonists and antagonists

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Converging lines of evidence indicate that nicotinic acetylcholine receptors (nAChRs) are involved in major depression. Epidemiologists suggest that smokers more often than non-smokers demonstrate depressive symptoms, and that depressed patients are less likely to cease smoking. In addition, depressed smokers are more dependent on cigarettes, and the cessation of smoking is often followed by a depressive episode [Covey et al., J Addict Dis, 1998; Glassman et al., JAMA, 1990; Lerman et al., Addict Behav, 1996]. Finally, smokers with a history of major depressive episode are more likely to relapse than smokes with no history of depression [Fergusson et al., Arch Gen Psychiatry, 1996, Kinnunen et al., J Clin Psychol, 1996]. Moreover, pharmacological lines of evidence indicate that the antidepressant drug, bupropion, antagonizes neuronal nAChRs [Slemmeret et al., J Pharmacol Exp Ther, 2000], and has also been used as the treatment for smoking cessation [Dewey et al., Synapse, 1999; Lief, Am J Psychiatry, 1996] It is also known that nicotine patches can improve the mood of depressed patients [Salin-Pascual et al., J Clin Psychiatry, 1996].

Based on these observations, it has been hypothesized that nicotine produces antidepressant effects, and that smokers "self-medicate" the underlying depressive illness with nicotine, and/or depressive symptoms produced by nicotine withdrawal [Markou et al., Neuropsychopharmacology, 1998]. Although this hypothesis is based on the assumption that nicotine may have antidepressant properties, the preclinical data on the antidepressant-like effects of nAChR ligands are ambiguous. For instance, in Ferguson et al., [Psychopharmacology (Berl), 2000] studies, nicotine appeared not to influence the learned helplessness response, though a subtype-selective nAChR agonist produced an antidepressant- like effect. Perhaps more suggestive are studies on the Flinders Sensitive Line rats, regarded as a "genetic animal model of depres-

sion". In these rats, which in drug-free state demonstrate an exaggerated immobility, acute or chronic administration of nicotine improved the performance in the forced swimming test [Tizabi et al., Psychopharmacology, 1999]. This effect was prevented by pretreatment with nAChR antagonist, mecamylamine (MEC), which by itself did not affect forced swimming response [Tizabi et al., Pharmacol Biochem Behav, 2000]. The most convincing study demonstrating antidepressant-like effect of nicotine was reported by Semba et al. [Biol Psychiatry, 1998] who showed that the chronic treatment with nicotine produced antidepressant-like effects in a learned helplessness "model of depression".

The lack of convincing preclinical data demonstrating that nicotine and/or nAChR antagonists produce antidepressant-like effects in the commonly used screening procedures (such as the forced swimming and tail suspension tests) is surprising. In the series of studies, our laboratory attempted to characterize the antidepressant-like effects of nicotine and nicotinic receptor antagonists after acute and long-term administration. We also investigated if nicotinic receptor ligands could affect the antidepressant-like effects of known antidepressants.

In the tail suspension test in C57/Bl male mice, nicotine (0.8–1.2 mg/kg *sc* or *ip*) given 15–60 min before the measurement exerted no effect on immobility [Popik et al., Br J Pharmacol, 2003]. However, nicotine significantly potentiated anti-immobility effects of citalopram and another antidepressant, imipramine. We further investigated if nAChR antagonists would influence the antidepressant-like effects of imipramine and citalopram. Unexpectedly, mecamylamine and dihydro-β-erythroidine also potentiated the antidepressant-like effect of imipramine. Mecamylamine but not dihydro-β-erythroidine also increased the antidepressant-like effect produced by citalopram. The interaction between nAChR antagonists and antidepressants appeared synergistic. Neither nAChR ligands, antidepressants nor combinations of the two, affected locomotor activity. These data suggest that although neither nicotine nor nicotinic receptor antagonists produce antidepressant-like effects per se, they could potentiate such effects of known antidepressants in the simple screening procedures after acute administration.

In the next series of experiments, we investigated the effects of sub-chronic and chronic treatment with imipramine, nicotine and their combination on the ability of a dopamine-mimetic challenge to produce locomotor stimulation as well as cortical density of β-adrenoceptors [Popik et al., Eur J Pharmacol, 2005]. One week of treatment with imipramine (10 mg/kg, twice daily) did not result in an altered response to the apomorphine (0.15 mg/kg) challenge, but after two weeks, the imipramine-treated rats demonstrated hyperactivity. Conversely, such increased locomotor response was observed in rats treated with nicotine (0.4 mg/kg, twice daily) for one but not for two weeks. Groups treated with nicotine + imipramine for one and two weeks demonstrated equally high hyperactivity in response to the apomorphine challenge. This effect was not different from the effects of one-week treatment with nicotine or two-week treatment with imipramine.

Neurochemical experiment demonstrated that the density of β-adrenoceptors was equally decreased by two (but not one) weeks of the treatment with imipramine, nicotine and their combination. These behavioral and neurochemical data suggested the antidepressant-like effect of the chronic treatment with nicotine, and an apparent discrepancy between the effect of nicotine and imipramine in a behavioral test (rapid though transient effect of nicotine, and slowly developing though sustained effect of imipramine) and neurochemical assay (nicotine acting as slowly as imipramine).

Altogether, these data support epidemiological reports indicating the antidepressant-like effects of nicotine, and suggest an involvement of nicotinic receptors in the effects of antidepressant drugs.

New vistas on the mode of action of antidepressants

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Since the discovery of the first antidepressants in the mid 1950s, the mechanism of their action has been the subject of intense research. Biological hypotheses of depressive disorders stated that deficits of monoaminergic transmission in the brain played an important role in the etiology of depression, and several classes of antidepressant drugs have been developed over years. However, though most currently used and clinically effective antidepressants exert their initial effects by increasing the synaptic levels of monoamines (serotonin and noradrenaline or dopamine), their clinical antidepressant effects are only observed after chronic administration. This suggests that a cascade of downstream effects is ultimately responsible for therapeutic efficacy of antidepressants.

Recent studies demonstrate that an impairment of neuroplasticity may underlie the pathophysiology of depressive disorders and the new hypotheses on the mechanism of action of antidepressants take into consideration their effects on neural plasticity and on remodeling of neuronal circuits. It is proposed that drugs of various classes have common antidepressant effects after chronic use because they may regulate transcription of the same set of downstream genes. Among candidates are genes encoding neurotrophic factors, including the brain-derived neurotrophic factor (BDNF) which is known for its long-term neurotrophic and neuroprotective effects, and is increased by treatments with various antidepressants. Recent data have shown that antidepressant drugs, besides their primarily pharmacological action on the availability of neurotransmitters, exert major effects on signaling pathways that regulate neuroplasticity and cell survival, such as the mitogen-activated protein (MAP) kinase and its downstream targets (e.g., the ribosomal S-6 kinase and the antiapoptotic protein, bcl-2). Although these pathways are regulated mainly by neurotrophic factors, the activity of MAP kinases can be also modulated by signals coming from several receptors for monoamines (i.e., serotonin and noradrenaline) as well as for other neurotransmitters (e.g. glutamate, neuropeptides and hormones) that are currently considered to play a role in the mechanism of action of antidepressant drugs. Thus, it is now assumed that several distinct receptor mechanisms trigger different but converging intracellular signal cascades that promote the expression of genes and proteins that play crucial role in restoring of neuronal functions.

The emergence of molecular neurobiology is now rapidly changing the traditional focus of antidepressant drug research with emphasis on effector sites beyond the receptors. Identification of such targets will advance future efforts towards the discovery of the next generation of antidepressants with a new mode of action in the brain.

Oral communications

Behavioral effects of citalopram and tianeptine in mice lacking the noradrenaline transporter

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Drugs which more or less selectively inhibit the neuronal reuptake of noradrenaline (NA), i.e., the noradrenaline transporter (NAT) inhibitors, are used in the treatment of depression. However, the role of serotonergic neurotransmission in the etiopathogenesis of depression as well as in the mechanism of action of antidepressant drugs (ADs) has been also strongly postulated. Recent development of genetically modified mice lacking the NAT [Xu et al., Nat Neurosci, 2000] opened new perspectives for studying the role of noradrenergic system in the mechanism of action of various ADs, including the drugs whose primary mode of action involves the serotonergic transmission.

In the present study, we investigated the responsiveness of the NAT knock-out mice ($NAT^{-/-}$) in the forced swimming test (FST) and tail suspension test (TST) upon the acute (single dose for FST and TST)) and subchronic (7 days for FST) treatment with the following ADs: citalopram (a selective serotonin reuptake inhibitor; 10 mg/kg) and tianeptine (a drug which is thought to enhance the reuptake of serotonin; 25 mg/kg)

Locomotor activity of untreated $NAT^{-/-}$mice, measured in photoresistor actometers, was significantly lower (ca. 30% reduction) as compared to the control WT mice. In the FST, on the other hand, untreated $NAT^{-/-}$ mice displayed significantly shorter (by 25–30%) immobility time than WT animals. Furthermore, in the TST the shortening of the immobility time in untreated $NAT^{-/-}$ mice was even more pronounced (ca. 45% reduction) compared to WT mice.

Acute treatment of WT mice with citalopram significantly shortened the immobility time in the TST, but tianeptine was without any effect in WT mice in this test. On the other hand, in $NAT^{-/-}$ mice subjected to TST, both citalopram and tianeptine significantly shortened the immobility time in comparison to the immobility time already diminished by the NAT knock-out itself.

The results obtained in the FST were different: citalopram did not significantly modify the immobility time of WT mice after acute (as well as subchronic) administration, whereas tianeptine administered acutely (and sub-chronically) shortened the immobility time in WT animals subjected to the FST. Acute administration of both drugs did not have any effect in $NAT^{-/-}$ mice, but upon subchronic administration the effects of both drugs were different: citalopram increased and tianeptine shortened the immobility time in the $NAT^{-/-}$ mice.

The significance of the obtained results in comparison of the data obtained previously with other ADs in $NAT^{-/-}$ mice will be a subject to further discussion.

Is the increase in BDNF mRNA expression an indicator of antidepressant efficacy?

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The hypothesis indicating that an increase in the BDNF expression is a common effect of antidepressant drug (AD) action has been widely accepted among neuro-psychopharmacologists. ADs administered chronically up-regulate BDNF mRNA and protein level. There are also reports that BDNF acts as an antidepressant in some behavioral tests. However, there is the increasing evidence that up-regulation of BDNF gene expression by ADs is less widespread across the treatments than previously thought.

In our laboratory, we have been using two approaches for investigating expression of genes – the microarray studies and *in situ* hybridization (ISH). We used these techniques in experiments with ADs as well as with other compounds. Since the results we have obtained for years, regarding the effects of ADs, were not consistent with current "neurotrophic" axiom, we were looking for biases in our methodology. But the increasing evidence indicating a complex regulation of BDNF mRNA by ADs have pushed us to look again at those data.

Here the effects of imipramine and desipramine on BDNF mRNA expression are presented. Additionally, we compared these data to the effects of cocaine, pilocarpine and chronic mild stress (CMS). Male Wistar rats weighing 200–250 g were used. In one experiment, rats were also subjected to the Porsolt test. Animals were usually decapitated 24 h after the last dose of the drug, brains were rapidly frozen for ISH studies or appropriate tissue (e.g. hippocampus) was collected for cDNA arrays. For microarray studies, total RNA was isolated from tissue using TRIZOL. QC was done by means of spectrophotometry (260/280 ratio > 1.8) and gel electrophoresis (morphological judgment of degradation). The best preparations were chosen for probe synthesis. [^{32}P]dCTP was used as a label. Autoradiograms of array membranes were obtained and digitalized using FujiBAS 5000 phosphoimager system. We used SuperArray Q series GEArray pathway specific arrays "Mouse Neurotrophins & Receptors". Data were analyzed by GEArray Analyzer software supplied by array manufacturer. For ISH, brains were sliced using cryostat and fixed in 4% formaldehyde, followed by acetylation (acetic anhydride in TEA buffer) and de-lipidation. Oligo: CTC CAG AGT CCC ATG GGT CCG CAC AGC TGG GTA GGC CAA GTT GCC TTG labeled with [^{32}S]-dATP with terminal transferase was used as a probe. Autoradiograms obtained with Kodak Biomax MR film were analyzed with the MCID system.

Imipramine administered repeatedly significantly reduced amount of mRNA coding for BDNF detected in the hippocampus either by microarray or ISH. We observed similar effect with desipramine. However, when animals receiving those ADs were subjected to the Porsolt test, the BDNF mRNA level was higher than in control animals. This is an interesting observation since the test could be regarded as stressful stimulus. These results are compared to those obtained with pilocarpine and CMS treatments which increased the level of BDNF mRNA in the rat hippocampus. The increase in the level of BDNF mRNA was also observed in the hippocampus of rats withdrawn from 5 daily doses of cocaine. An interesting finding is, however, that animals treated likewise behaved as "depressed" in the Porsolt test.

Reviewing the recent publications concerning the regulation of BDNF expression by ADs one could say that valid axiom indicating that ADs act *via* up-regulation of BDNF expression is not so obvious any more. There is growing evidence that neurotrophin theory of depression, although very stimulating, is not the final answer to antidepressant action mystery.

Biochemical and molecular modeling study focused on stereoselectivity of 8-OH-DPAT toward the serotonin 5-HT_{1A} receptor

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During the last 25 years, 8-OH-DPAT was extensively used for pharmacological research to evoke variable effects of 5-HT_{1A} receptor activation. Although 8-OH-DPAT is a stereoselective compound, the great majority of pharmacological investigations has been performed using racemic 8-OH-DPAT. Therefore, the biochemical as well as behavioral profiles of both 8-OH-DPAT enantiomers are not circumstantiated. The biochemical study was designed to investigate the possible difference between racemic 8-OH-DPAT and its R-isomer at the presynaptic 5-HT_{1A} receptors, hence, capability of racemate (0.05, 0.1 mg/kg *sc*) and its active counterpart R-8-OH-DPAT (0.05, 0.1 mg/kg *sc*) to influence 5-HT synthesis rate in rat prefrontal cortex, hypothalamus, hippocampus and brainstem was evaluated by HPLC/ED technique. Similar effects of R-enantiomer and racemate were observed in all tested brain areas apart from the hypothalamus.
Furthermore, molecular modeling studies were undertaken in order to explain the phenomena of the stereostructure-key to the intrinsic activities of R- and S-8-OH-DPAT toward 5-HT_{1A} receptor. A reliable 3D model of the rat 5-HT_{1A} receptor was used to study the interactions with both R- and S-enantiomers of 8-OH-DPAT, estimation of their binding affinities and free energies of binding to the receptor. Moreover, the analysis of possible differences in their binding modes was performed. Docking studies and the dynamics of ligand-receptor complexes revealed similarities of the free energy of binding and affinity of R- and S-8-OH-DPAT for the 5-HT_{1A} receptor. Simultaneously, a significant difference between the structures of receptor-ligand complexes (especially within the part of the domain responsible for G-protein coupling) after molecular dynamics simulations was observed, which may contribute to the differences in efficacy of both 8-OH-DPAT enantiomers. The results of both biochemical and exhaustive computational studies emphasized the stereoselectivity of 8-OH-DPAT toward the 5-HT_{1A} receptor.

Neonatal lesion of the noradrenergic system modifies anxiety-like behavior and GABAergic neurotransmission in the prefrontal cortex of adult rats

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DSP-4 [(N-2-chloroethyl)-N-ethyl-2-bromobenzylamine] selectively damages noradrenergic projections originating from the locus coeruleus. The studies performed previously in our laboratory confirmed that the application of DSP-4 to newborn rats on the first and third day after birth caused the lesion of noradren-

ergic fibers in the frontal cortex and the hippocampus with a simultaneous brain stem and cerebellum hyperinnervation and that these processes are long-lasting. In the present research, we applied this model to investigate the interactions between noradrenergic and GABAergic systems.

The noradrenergic system is important in manifestations of anxiety, depression, sleep, seizure activity and others. Controversially, several lines of evidence suggest that the fast-inhibitory activity of GABA is mediated by the $GABA_A$ receptor and is responsible for the regulation of vigilance, anxiety, muscle tension, epileptogenic activity and memory functions. Lack of the balance between excitatory neurotransmission and GABA-mediated inhibition contributes to many serious perturbations of the brain function. Taking into consideration the interactions between these two systems, the aim of the study was to investigate the effect of the damage of the central noradrenergic system in the early period of the development of rats on alterations of the function of the central GABAergic system in adult animals.

The examination of the GABA-NA interaction in neonatally lesioned rats let us better understand the compensatory processes which appear during the neurogeneration, during the development of the "damaged" brain. Therefore, we decided to conduct two anxiolytic tests (plus maze test and social interaction test) in control and DSP-4-lesioned rats after the application of diazepam (DZP, 5.0 mg/kg *ip*). Furthermore, we estimated the level of GABA in the frontal cortex, hippocampus, brain stem and cerebellum after the injection of DZP (5.0 mg/kg *ip*) and vigabatrin (VGB, 500.0 mg/kg *ip*). To assess the biochemical processes which run in the rat's brain, we decided also to investigate the influence of the neonatal noradrenergic lesion on the extracellular GABA level in the prefrontal cortex of adult rats after the application of VGB (500.0 mg/kg *ip*) using *in vivo* microdialysis.

The results of the presented studies indicate that the damage of the central noradrenergic system of newborn rats changes the reactivity of the central $GABA_A$ receptors and at the same time modifies the metabolism of GABA in the prefrontal cortex of adult rats.

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MTEP-induced adaptation of adrenergic receptors

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MTEP, 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine is a novel, highly selective and non-competitive mGluR5 receptor antagonist. The antidepressant-like activity of MTEP was evaluated in behavioral tests and in experimental models of depression (the forced swim test, the tail suspension test, olfactory bulbectomy model of depression; [Pałucha et al., Drug News Perspect, 2005]).

Long-term treatment with conventional antidepressant drugs and electroconvulsive shocks led to development of many adaptive changes in the central nervous system. The most commonly demonstrated changes are β_1-down-regulation, α_1-up-regulation and α_2-down-regulation.

The aim of this study was to investigate the adaptive changes in noradrenergic receptors following chronic treatment with MTEP in mice. Male albino Swiss mice were housed in groups of 9–10 with free access to food and water. MTEP (1 mg/kg, *ip*) was administered once daily for 14 days. Twenty-four hours after the last administration, the animals were decapitated, their brains were removed, and cortices (neocortex) were dissected and immediately frozen over

solid CO_2. The frozen tissue was stored at –80°C for 2–6 weeks before the assay. The radioligand binding to β_1-, α_1-, α_2-adrenoceptors was performed in mouse cortex using [^{3}H]CGP 12177, [^{3}H]prazosin and [^{3}H]clonidine as radioligands, respectively.

Two-week administration of MTEP significantly increased the specific binding (by 53% at 0.2 nM and by 41% at 1 nM, $p < 0.05$) to α_1-adrenoceptors. The reduction of specific binding to β_1-adrenoceptors following MTEP did not reach statistical significance. Specific binding to α_2-adrenoceptors was not affected by MTEP treatment.

These preliminary results show selective effect of chronic administration of MTEP (an antagonist of mGluR5) on α_1-adrenergic receptor. The effect on β_1-adrenoceptors needs further evaluation.

The data suggest some similarities between MTEP and antidepressant drugs in affecting α_1-adrenergic receptors and further support potential antidepressant activity of group I mGlu receptors.

Effect of chronic antidepressant drugs and zinc treatment on synaptic hippocampal zinc concentration in rats

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Electroconvulsive seizures (ECS), one of the most potent and rapid-acting therapies available for treating depression, induce mossy fiber sprouting (when assayed by means of synaptic zinc method), and this indicates an increase in the synaptic zinc level in the hippocampus following such therapy [Vaidya et al., Neuroscience, 1999; Gombos et al., Brain Res, 1999].

Zinc is an endogenous neuromodulator of glutamate (mainly N-methyl-D-aspartatic acid – NMDA) receptors which may be involved in the psychopathology and treatment of depression [Nowak et al., Pharmacol Rep, 2005]. This trace biometal is present in the whole body, but the brain (especially the hippocampus and cerebral cortex) is the organ with the highest zinc concentration [Frederickson et al., Prog Brain Res, 1990]. The synaptic zinc pool represents about 5% of the total zinc in the brain, and neurons that sequester zinc in the presynaptic vesicles are named zinc-containing neurons. This pool of zinc can be selectively stained by several histochemical procedures and available to histological and histochemical studies [Danscher et al., Histochemistry, 1982; Frederickson et al., Prog Brain Res, 1990]. The aim of the present study was to investigate the effect of chronic zinc hydroaspartate and antidepressant drug (imipramine and citalopram) administration on the synaptic zinc pool in the rat hippocampal slices, by using zinc-selenium method.

The experiments were carried out on male Wistar rats. Zinc hydroaspartate (Farmapol, Poznań, Poland) at two doses: 65 mg/kg (11.5 mg of zinc/kg) and 10 mg/kg (1.8 mg of zinc/kg), imipramine (20 mg/kg), citalopram (20 mg/kg) or vehicle (0.9% sodium chloride, control rats) were administered intraperitoneally, once daily for 14 days. Sodium selenite was administered *ip* 24 h after the last administration of drugs.

Our results indicate that chronic zinc hydroaspartate *ip* administration at a dose 65 mg/kg increases the pool of synaptic zinc in the majority of rat hippocampal areas (by 72–190%), except for the stratum moleculare and stratum radiatum CA, and perforant path DG. This effect is similar to that observed following chronic ECS treatment (Lamot et al., Brain Res, 2001). In contrast, no changes were found after zinc hydroaspartate at the lower dose (10 mg/kg) and imipramine or citalopram treatment.

This data suggest that the increase in the synaptic zinc level is not a common phenomenon of antidepressant agents.

Zinc-induced adaptation in adrenergic receptors

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Zinc, a trace biometal, is involved in many biological mechanisms in living organisms [Fredericson, 1989]. Recent studies demonstrated antidepressant-like activity of zinc in animal tests and models of depression [Nowak et al., 2001; 2002; 2003; Rosa et al., 2003]. Moreover, preliminary clinical data suggest beneficial role of zinc supplementation in antidepressant therapy in unipolar depression [Nowak et al., 2003]. However, the molecular mechanism of antidepressant zinc action remains still unclear.

Chronic treatment with pharmacological antidepressant therapy and electroconvulsive shocks induces adaptive changes in the CNS. The most common changes are: β_1-down regulation, α_2-down regulation and α_1-up regulation.

The aim of the study was to investigate the adaptive changes in noradrenergic receptors after chronic zinc treatment. The experiments were performed on male albino Swiss mice. Zinc hydroaspartate (Farmapol, Poznań, Poland) at the dose 65 mg/kg (11.5 mg of zinc/kg) was administrated intraperitoneally once daily for 14 days. Control animals received a vehicle solution (0.9% sodium chloride). Twenty-four hours after the last administration, the animals were decapitated, their brains were removed, and cortices (neocortex) were dissected and immediately frozen over solid CO_2. The frozen tissue was stored at –80°C for 2–6 weeks before the assay. The radioligand binding to β_1-, α_1-, α_2-adrenoceptors was performed in mouse cortex using [^{3}H]CGP 12177, [^{3}H]prazosin and [^{3}H]clonidine, respectively.

Chronic zinc hydroaspartate administration significantly increased ligand binding to α_1-adrenergic receptors (to 146% at 0.2 nM and to 133% at 1 nM, $p < 0.05$). Moreover, zinc treatment decreased the ligand binding to β_1-adrenergic receptors to 60% (statistically significant only at 0.2 nM [^{3}H]ligand concentration, $p < 0.05$). In contrast, zinc treatment did not significantly affect binding to α_2-adrenergic receptors.

These preliminary results show effects of chronic zinc treatment on α_1- and β_1-adrenergic receptors.

The data demonstrate some similarities between zinc and standard antidepressant therapy in affecting adrenergic receptors (α_1 up-regulation, β_1 down-regulation), and further support potential antidepressant activity of zinc.

Effects of single and repeated administration of electroconvulsive shock on some parameters of the activity of peritoneal macrophages

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Depressive disorders are associated with abnormal functions of the immune system and immunosuppression as well as pathological activation of some indices of immunoreactivity were reported [Maes, Prog Neuropsychopharmacol Biol Psychiatry, 1995]. The excessive activity of monocytes and macrophages observed in depression is of great interest in view of the fact that their products, i.e. proinflammatory cytokines, induce depressive-like symptoms in both humans and animal models [Wichers et al., Int J Neuropsychopharmacol, 2002; Leonard et al., Int J Neuropsychopharmacol, 2002]. Previously, we reported that repeated administration of electroconvulsive shock (ECS) to rats reduced the ability of their macrophages to produce nitric oxide (NO) if assessed 24 h after the last treatment [Roman et al., J ECT, 2005]. Here, we investigated the effects of single and repeated administration of ECS on some chosen parameters of the activity of peritoneal macrophages.

Male Wistar rats weighting 250–280 g were kept under standard animal house conditions. ECS (150 mA, 50 Hz, 0.5 s) was generated by the GE-01 apparatus and was administered through ear clips, singly or repeatedly once daily through 10 consecutive days. Control animals received sham-ECS (without electric impulses), single or tenfold, respectively. Twenty four hours after the last treatment, rats were sacrificed by decapitation. Macrophages were eluted from peritoneal cavity with cold PBS buffer, counted and resuspended in culture medium, and placed on culture plates at 1×10^5 cells per well. After initial (3 h) incubation, the following parameters were assessed: the ability to reduce Alamar Blue [O'Brien et al., Eur J Biochem, 2000] or MTT [Liu et al., J Neurochem, 1997], spontaneous and PMA-stimulated synthesis of superoxide anion (O_2^-) by reduction of NBT [Pick, Methods Enzymol, 1986], adherence by crystal violet staining [Kueng et al., Anal Biochem, 1989], and pinocytosis of neutral red [Płytycz et al., Folia Biol (Kraków), 1992]. In separate cultures, the spontaneous and LPS-stimulated NO production after 36 h of incubation was investigated in supernatants [Marcinkiewicz et al., Arch Immunol Ther Exp (Wrocław), 1994] and the remaining cells were assessed for their ability to reduce Alamar Blue or MTT. Cell deaths were also examined with LDH test after 36-h incubation period [Decker et al., J Immunol Methods, 1988].

We found statistically significant changes in biological properties of macrophages that appeared after 36 h of incubation, especially in cultures stimulated with LPS, while no differences between groups assessed after 3 h of initial incubation were observed. The marked, but statistically insignificant, decrease (by 30% and 15% in unstimulated and stimulated cultures, respectively) of the NO synthesis in rats receiving tenfold ECS treatment in comparison to respective controls was not connected to the disturbances of macrophage vitality. However, the decrease in NO synthesis was accompanied by the substantial increase in the macrophages' metabolic activity reflected by their ability to reduce Alamar Blue or MTT.

Our results suggest that chronic treatment with ECS may induce long-lasting changes in the peritoneal macrophages' activity. The attenuation of their proinflammatory properties, as manifested by the different redox status of cells in the 36 h culture, suggests that ECS may change the primarily immunoregulatory functions of macrophages.

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Effects of chronic administration of corticosterone on anxiety-like behavior and c-Fos expression in the brain structures

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The aim of this study was to examine changes in rat emotional behavior and to find the brain structures which are involved in the mediation of behavioral effects related to the chronic administration of glucocorticoids. The effects of acute and chronic pretreatment of rats with different doses (5 and 20 mg/kg) of corticosterone were analyzed in two models of anxiety: neophobia-like behavior in the open field test, and freezing reaction in the conditioned fear test. Behavioral effects were compared with changes in blood corticosterone concentration and expression of immediate early gene (c-Fos) in brain structures involved in the regulation of fear, anxiety and feedback control of the hormone stress response. It was found that acute administration of corticosterone enhanced rat exploratory behavior, and decreased freezing reaction. On the other hand, chronic pretreatment with corticosterone inhibited exploratory behavior, enhanced freezing responses, decreased plasma corticosterone concentration, and caused a complex pattern of changes in c-Fos expression stimulated by exposure of rats to the aversively conditioned context. Chronic corticosterone administration attenuated neuronal activity in the magnocellular neurons of the hypothalamic paraventricular nucleus and dentate gyrus, enhanced c-Fos expression in the primary motor cortex (M1), and central nucleus of the amygdala (CeA), in comparison to control animals not subjected to contextual fear test. In conclusion, the present data suggest that chronic corticosterone treatment increases the activity of M1 and CeA with subsequent improvement of memory of aversive events, and simultaneously stimulates a negative feedback mechanism in the paraventricular nucleus leading to the decrease in blood corticosterone concentration.

Influence of stimulation of group III mGluRs by ACPT-1 on the parkinsonian-like akinesia and activity of striatal output neurons in rats

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The aim of the present study was to determine whether ACPT-1 (an agonist of group III mGluRs), administered into the striatum, GP or SNr was able to (1) reverse the haloperidol-induced catalepsy in rats;

(2) modulate the functions of striatal output GABAergic neurons measured by proenkephalin (PENK), prodynorphin (PDYN) and glutamate decarboxylase (GAD67) mRNAs expression in the striatum.

Haloperidol (0.5 mg/kg *ip*) induced strong catalepsy in rats. ACPT-1 injected bilaterally into the rostral striatum (0.4 to 1.6 μg/0.5 μl/side) significantly attenuated the haloperidol-induced catalepsy. The most pronounced anticataleptic effect of ACPT-1 (0.1 to 1.6 μg/0.5 μl/side) was obtained after its injection to the globus pallidus (GP). In the SNr, only the highest dose of ACPT-1 (1.6 μg/0.5 μl/side) evoked anticataleptic effect. ACPT-1 given alone did not induce any catalepsy in rats. Consistent with the behavioral data, haloperidol (3 × 1.5 mg/kg *sc*) significantly increased PENK and GAD67 mRNAs expression in the striatum, whereas PDYN mRNA levels were not affected by this treatment. ACPT-1 injected bilaterally into the striatum (3 × 1.6 μg/0.5 μl/side) significantly attenuated the haloperidol-increased PENK mRNA expression, but had no effect on the increased GAD67 mRNA expression. ACPT-1 administered into the GP or SNr influenced neither the haloperidol-increased striatal PENK mRNA nor GAD67 mRNA expression. Interestingly, this compound increased PENK mRNA expression when given alone into the GP.

Our results demonstrate that stimulation of group III mGluRs in the striatum, GP and SNr may exert antiparkinsonian-like effect in rats. Anticataleptic effect of ACPT-1 after intrastriatal injections may be related to normalization of function of the striatopallidal pathway.

Influence of the subchronic administration of a pesticide paraquat on the dopaminergic system in rats

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Paraquat (PQ) is a toxin suggested to contribute to pathogenesis of Parkinson's disease. Our recent study [Ossowska et al., Eur J Neurosci, 2005] has shown that a long-term PQ administration produces a slowly developing loss of dopaminergic neurons in the substantia nigra (SN) and delayed deficits of dopaminergic transmission in the caudate-putamen (CP) which may resemble processes progressing in Parkinson's disease.

In the present study, we examined the effects of subchronic treatment with PQ (5 days, one injection per day, 2–3 days of withdrawal) on the number of TH immunoreactive (THir) cells in the SN, the levels of dopamine (DA) and its metabolites in the CP and SN, as well as the binding of [^{3}H]GBR 12.935 to dopamine transporter (DAT) in the CP. A level of proenkephalin (PENK) mRNA in the CP as a marker of the GABAergic striatopallidal neurons was measured as well.

We observed a 22% loss of the number of THir cells in the SN after PQ administration. The levels of the DA metabolites (DOPAC and HVA) in the CP dropped by 35% and 44%, respectively, along with the DA turnover rates – DOPAC/DA (54% loss), HVA/DA

(52% loss) and 3-MT/DA (37% loss). The binding of [^{3}H]GBR 12.935 to DAT decreased by 13–25%. The PENK mRNA level was lowered by 23–33%.

The above results seem to suggest that PQ administered subchronically induces a modest degeneration of dopaminergic nigrostriatal neurons, inhibits the striatal dopaminergic transmission and functioning of the GABAergic striatopallidal neurons.

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Role of nitric oxide in amphetamine-induced biogenic amine release into brain microdialysate of adult rats prenatally exposed to lead

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Lead (Pb) is a highly neurotoxic agent in both mammals and humans. It is a major contaminant of the anthropogenic environment, due to its high natural abundance, massive industrial use and being a component of paints and a pollutant of crude gasoline. Lead is transported easily *via* the circulation and readily crosses the blood-brain barrier. Many studies have demonstrated that exposure to environmental lead adversely affects a variety of neurotransmitter systems including dopaminergic, noradrenergic, cholinergic and GABAergic, suggesting that this harmful pollutant affects the central nervous system. The developing brain of mammalians is more susceptible to the toxic effect of lead, as compared to mature brain tissue, due to a greater uptake of the lead by fetal brain, which is a result of slow development of blood-brain barrier at this stage.

In this study, the effect of lead exposure during pregnancy on amphetamine-induced biogenic amines release into microdialysate of the brain striatum and frontal cortex of adult rats, and role of nitric oxide (NO) in this process were investigated. Pregnant Wistar rats consumed 250 ppm of lead [$Pb(CH_3COO)_2$ $3H_2O$] in their drinking water throughout their entire pregnancies. On the day of parturition, the lead-containing water was replaced by tap water, and the offsprings remained with their mothers for 21 days. Control pregnant rats consumed water without the metal. Adult male pups from both groups (lead-exposed and control) were pretreated with 7-nitroindazole 10.0 mg/kg *ip* or saline 1.0 ml/kg *ip* and 30 min later were injected with amphetamine 1.0 mg/kg *ip* or saline 1.0 ml/kg *ip*, and *in vivo* microdialysis study in the striatum and frontal cortex was performed. Using a Couluchem II data analysis system (ESA) to analyze monoamines and metabolites in the *in vivo* microdialysates, contents of DA, DOPAC, HVA, 5-HT, 5-HIAA, NA were estimated [Nowak et al., Pol J Pharmacol, 2001].

It was shown that amphetamine increased biogenic amines release into microdialysates depending on the examined group (lead-exposed or control) and the brain structure. 7-Nitroindazole modified the effect of amphetamine on biogenic amines release into microdialysate showing that nitric oxide participates in the above action.

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Effect of simultaneous ontogenetic lesion of noradrenergic and dopaminergic neurons on 5-hydroxytryptamine level in the brain and reactivity of dopamine receptors in rats

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N-(2-chlorethyl)-N-ethyl-2-bromobenzylamine (DSP-4), an alkylating neurotoxin selective for noradrenergic neurons, crosses the blood-brain barrier and produces long-lasting noradrenergic denervation [Jaim-Etcheverry et al., Trends Pharmacol Sci, 1983].

Another common neurotoxin, 6-hydroxydopamine (6-OHDA), unable to cross the blood-brain barrier but often administered into the lateral ventricles (*icv*) of the brain, is frequently used to destroy dopaminergic neurons [Jacobowitz et al., Pharmacol Rev, 1974], while simultaneously inducing sprouting and hyperinnervation of the brain by serotoninergic fibers [Brus et al., J Pharmacol Exp Ther, 1984].

In this study, the reactivity of the central dopamine receptors was examined in adult rats lesioned as neonates, simultaneously with DSP-4 and 6-OHDA. Newborn male Wistar rats were injected with DSP-4 (50 mg/kg *sc* twice: on the day of birth and 3rd day of life) [Nowak et al., Pharmacol Rep, 2005] and on the 3rd day of life with 6-OHDA (135 g *icv*, half into each lateral ventricle) [Brus et al., J Pharmacol Exp Ther, 1994]. Control newborn rats were injected with saline (*sc*, *icv*). At 8–10 weeks, the following studies were performed: (a) analysis of biogenic amines and their metabolites (NA, MOPEG, DA, DOPAC, HVA, 3-MT, 5-HT, 5-HIAA) in the striatum, frontal cortex, hippocampus and cerebellum; (b) behavioral testing (irritability, locomotion, yawning, exploratory activity, oral activity, stereotyped behavior, catalepsy and others) after treatment with dopamine receptor agonists (7-OH-DPAT, SKF 38393, quinpirole, apomorphine) and antagonists (haloperidol, SCH 23390).

We have found that NA levels in the hippocampus and frontal cortex of adult rats injected with DSP-4, *sc* twice, as neonates were significantly reduced, but increased in the cerebellum. No changes in DA and other amine levels were observed. 6-OHDA applied to newborn rats *icv* on the 3rd day significantly decreased DA and DOPAC levels in the striatum and frontal cortex, and increased 5-HT and 5-HIAA in the striatum of adult rats, as compared to the control. Simultaneous injection of DSP-4 and 6-OHDA to newborn rats decreased NA, DA, DOPAC in the brain and increased 5-HT and 5-HIAA in the striatum to a higher extent as compared to the control and 6-OHDA alone lesioned rats. Simultaneous lesion of rats, as neonates, with DSP-4 and 6-OHDA resulted in the enhanced agonist and antagonist action on assorted behavioral parameters, as compared to control, DSP-4 (alone)- and 6-OHDA (alone)-treated groups.

From the above, we conclude that simultaneous lesion of the central noradrenergic and dopaminergic system in neonatal rats modified reactivity of dopamine receptors to a greater extent than after a 6-OHDA lesion alone. In these rats the central serotoninergic system may play a role in altered behavioral responses, as serotonin levels are increased in the brain, estimated by biochemical method.

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Involvement of calcineurin, nNOS and $cPLA_2$ in the cytoprotective effect of FK506 and cyclosporin A on astrocytes exposed to simulated ischemia *in vitro*

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One of the hypotheses concerning cellular neuroprotective mechanisms of FK506 and cyclosporin A (CsA) action is that these compounds act through calcineurin (CaN) inhibition, thereby preventing dephosphorylation/activation of neuronal NO-synthase (nNOS) and subsequent NO mediated toxicity. A link to inhibition of $cPLA_2$ was also suggested since attenuation of free fatty acid liberation was supposed to prevent the assembly of an mitochondrial permeability transition pore (MPTP), leading to a decreased mitochondrial membrane permeability, and coupling respiration and oxidative phosphorylation.

The present study is focused on mechanisms involved in the protective effect of FK506 and CsA against ischemic injury of astrocytes *in vitro*. We investigated whether this effect might be mediated through attenuation of $cPLA_2$ activity and whether attenuation of CaN or nNOS in ischemic astrocytes is also involved in their anti-apoptotic action. Cell viability, antioxidant response and mitochondrial transmembrane potential (MTP) were determined as markers of astrocyte metabolism.

On the 21st day *in vitro,* cultures of astrocytes were subjected to ischemia-simulating conditions (92% N_2, 5% CO_2, 3% O_2 at 37°C) for 8 h and exposed to FK506 (10–1000 nM) and CsA (0.25–10 μM).

The obtained data suggest the cross-talk between the action of 0.25–1 μM CsA as well as 1 μM FK506 on CaN, nNOS and $cPLA_2$ expression in anti-apoptotic signal transduction pathways. Moreover, we have shown that immunosuppressants at these concentrations protected glial cells against ischemia-induced apoptosis through the increase in cell viability, mitochondrial function restoration and attenuation of oxidative stress. Finally, in our study low concentrations of FK506 (10 nM and 100 nM) exerted limited effect on the parameters under study. These results indicate that FK506 and CsA might act as protective agents through different mechanisms on ischemic astroglial cells, and effect of FK506 strongly depends on the concentration used.

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Effect of angiotensin II and IV on ERK1/2 and CREB activation in the rat astroglial cells

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Angiotensin peptides activate several transcription factors and signaling molecules that results in cell growth alteration and blood pressure changes, and influences memory. A stimulus-induced transcription factor, the cAMP response element binding protein (CREB) and extracellular signal-regulated protein kinase 1/2 (Erk1/2) were recently identified as potentially important signaling molecules in memory formation. In this study, we evaluated the effect of angiotensin peptides on astroglial cell growth, apoptosis, oxidative stress, intracellular calcium, CREB expression and its phosphorylation at Ser133 and ERK1/2 expression and phosphorylation at Thr202/Tyr204. Ten days old cultures of the rat astroglial cells grown in 10 cm diameter surface-modified polystyrene Falcon Primaria dishes were treated with angiotensin II (Ang II; 10 μM final concentration), angiotensin IV (Ang IV; 10 μM final concentration) or both in the presence or absence of AT1 receptor antagonist losartan (100 μM final concentration), AT2 receptor antagonist PD123319 (100 μM final concentration) and/or protease inhibitor-bestatin (100 μM final concentration) for 24 h. The treatment did not significantly affect cell proliferation, apoptosis and oxidative stress as determined by cell cycle flow cytometry assay (propidium iodide fluorescence), phosphatidylserine externalization (annexin V) and dichlorofluorescein (DCFDA) fluorescence, respectively. Intracellular calcium (fura 2 fluorescence) was increased almost 2-fold in cells treated with Ang II but not with Ang IV and this effect was partly abolished by losartan. Ang II increased CREB expression (immunoblot) and to a lesser extent ERK1/2 and phosphorylated ERK1/2 expression (ELISA), while Ang IV significantly enhanced both ERK1/2 forms. These changes were partly abolished by losartan but not by PD123319 or bestatin indicating that effect of Ang II on astroglial cells could be coupled to the up-regulation of CREB and ERK1/2 functionality most probably *via* activation of AT1 receptors.

Inhibitory effects of dehydroepiandrosterone and pregnenolone on necrotic and apoptotic neuronal cell damage

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Neuroactive steroids show protective properties in some models of neurodegeneration. Previously, we found that some neuroactive steroids significantly protected SH-SY5Y cells against free radical-related damage. However, their involvement in regulation of apoptotic process has not been elucidated. Therefore, in the present study, effects of dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS) and pregnenolone (PGL) on staurosporine-induced caspase-3 activity and mitochondrial membrane po-

tential in SH-SY5Y human neuroblastoma cells have been evaluated. Staurosporine (1 μM) present in cell culture for 24 h enhanced the caspase-3 activity by ca. 600%. Pretreatment with DHEA (0.1–100 μM), DHEAS (0.1–100 μM) and PGL (0.01–1 μM) attenuated the staurosporine-induced caspase-3 activity in a concentration-dependent manner. On the other hand, pregnenolone sulfate and allopregnanolone inhibited the staurosporine effects but only at the lowest concentration. Moreover, the neuroactive steroids prevented also the staurosporine-evoked decrease in the mitochondrial membrane potential. These results indicate that neuroactive steroids may exert their neuroprotective effects through inhibition of mitochondrial pathway of apoptosis.

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Baclofen and hypoxia influence MMP-2 and MMP-9 in the rat hippocamus

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Matrix metalloproteinases (MMPs) are extracellular proteases that have recognized roles in cell signaling, plasticity, long-term potentiation and memory processes [Dzwonek et al., FEBS Lett, 2004; Kaczmarek et al., J EMBO, 2002; Szklarczyk et al., J Neurosci, 2002; Wright et al., Peptides, 2002]. Their activation and function are instrumental for pathological and physiological processes in the brain [Jourguin et al., Eur J Neurosci, 2005; Nagy et al., J Neurosci, 2006]. The results of Zalewska et al. [Acta Neurobiol Exp, 2002] have shown that MMP pathway may function as a component of delayed neuronal death cascade in the apoptogenic CA1 sector of the hippocampus after transient global ischemia. The hippocampus damage induced by hypoxia is probably responsible for impairment of the consolidation process observed in our previous study [Car et al., Pharmacol Res, 2001]. However, baclofen, an agonist of $GABA_B$ receptors, impaired acquisition and enhanced anxiety, it also provided protection against hypoxia-induced impairment of acquisition and consolidation in the passive avoidance, as well as reduced anxiety of rats after induction of hypoxia [Car et al., Pharmacol Res, 2001].

In the present study, we focused on the measurement of MMP-2 and MMP-9 in the rat hippocampus after activation of $GABA_B$ receptors by baclofen in rats that had undergone short-term hypoxia. Baclofen reduced total and active form of MMP-2. This agonist did not influence all tested forms of MMP-9. Gel zymography showed elevations in the MMP-9 proform activity in the hippocampus 30 min after hypoxia induction. Baclofen did not affect the hippocampal MMP2 and MMP-9 activity in rats that had undergone hypoxia. Hypoxia enhanced effect of baclofen on MMP-2 (total and active form).

Concluding, the enhanced production of MMP-9 after hypoxia is compatible with the results described by Panas et al. [Neurobiol Disease, 2001] and Lee et al. [J Neurosci, 2004] but the present work showed for the first time a very early MMP-9 induction observable after 30th minute of hypoxia. The effect of baclofen on MMP-2 may suggest the role of $GABA_B$ receptor in reconfiguration of extracellular matrix in the hippocampus of rats. Here, we explored a possible role for MMP-2 in baclofen activity.

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Developmentally regulated vulnerability of neocortical neurons to excitotoxic and apoptotic stimuli and neuroprotective role of memantine

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Susceptibility of neuronal tissue to toxic agents seems to depend on its stage of maturation. In order to further elucidate this problem, we investigated NMDA-evoked toxicity and staurosporine-induced apoptosis in primary neocortical neurons at various stages of development *in vitro*. Additionally, the effect of clinically useful NMDA receptor antagonist, memantine on the above processes was evaluated. The biochemical measurement of caspase-3 activity and DNA fragmentation detected by Hoechst 33342 staining were employed to estimate apoptotic changes in cell cultures and the lactate dehydrogenase (LDH) release assay was used to assess scale of cell death. The obtained data showed that immature neurons (7 DIV) were more vulnerable to staurosporine-induced apoptosis and, on the other hand, were more resistant to NMDA toxicity than the mature ones (12 DIV). Memantine (0.1–2 μM) attenuated staurosporine-induced LDH release and caspase-3 activity only in 7 DIV neurons by about 20–25% and 40%, respectively. NMDA (200 μM) was able to increase LDH release by about 70% of control value only in 12 DIV neurons, and these changes were partially attenuated by memantine. Interestingly, NMDA did not affect survival of 7 DIV cells but attenuated staurosporine-induced LDH release and caspase-3 activation in those neurons to similar extent as memantine did. All in all, these results point to the developmentally regulated effects of NMDA receptor ligands, with profound neuroprotective effects of both, NMDA and memantine in immature neocortical neurons and neurotoxic effect of NMDA in mature ones.

Effects of $GABA_B$ receptor ligands on the "depressive-like" behavior in rats withdrawn from cocaine self-administration

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It is well-documented that withdrawal from drugs of abuse in humans induces symptoms that appear isomorphic to those of a major depressive disorder. In preclinical studies, discontinuation of repeated treatment with abused drugs induces "depressive-like" behavioral changes, i.e. increases immobility time in the forced swim test (FST) [Barr et al., Neurosci Biobehav Rev, 2005]. Recent findings point to the role of γ-amino butyric acid (GABA) in the pathology of depression and in the mechanism of action of antidepressant drugs [Cryan et al., Trends Parmacol Sci, 2005].

In the present study, we investigated the effect of $GABA_B$ receptor agonists baclofen and SKF 97541 and the $GABA_B$ receptor positive modulator CGP

7930 on immobility in the FST in either naive rats or animals withdrawn from cocaine self-administration.

To this end, male Wistar rats were trained to self-administer cocaine (0.5 mg/kg/infusion) under a fixed ratio (FR) 5 schedule of reinforcement in 2-h daily sessions (Monday–Saturday) and then were withdrawn from cocaine.

When given to naive rats, baclofen (0.25 mg/kg, *ip*), SKF 97541 (0.01–0.02 mg/kg, *ip*) or CGP 7930 (1–3 mg/kg, *ip*) significantly decreased immobility time in the FST. None of the investigated drugs altered climbing or swimming behaviors. In rats withdrawn from cocaine self-administration (0.5 mg/kg/infusion), we observed a significant increase (by 43%) in immobility time and a tendency to decrease climbing on the 3rd (but not on 1st, 10th or 30th) extinction day. In those rats, baclofen (0.125 mg/kg) induced a significant decrease (by 53%) in immobility time and a tendency to increase climbing and swimming behaviors. Either SKF 97541 (0.005 mg/kg) or CGP 7930 (0.3 mg/kg) showed a tendency to decrease immobility time, without alterations in climbing and swimming behaviors.

Our results indicate that $GABA_B$ receptor agonists and modulators produce effects that are characteristic of antidepressant drugs. Moreover, they seem to counteract the "depressive-like" effect in rats withdrawn from cocaine self-administration. These findings may suggest the therapeutic potential of $GABA_B$ receptor agonists in the treatment of cocaine withdrawal symptoms.

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Effects of serotonin $(5\text{-HT})_{1B}$ receptor ligands on cocaine or "natural" (food) reinforcement and relapse in rats

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Numerous data indicate that dopamine-dependent cocaine behavioral responses can be modified by serotonin (5-HT) neurotransmission [Filip et al., Pharmacol Rep, 2005]. Recent findings suggest that stimulation of 5-HT_{1B} receptors may influence the maintenance of cocaine self-administration [Parsons et al., J Neurosci, 1998] and reinstatement of drug-seeking behavior [Acosta et al., Pharmacol Biochem Behav, 2005].

In the present study, we used the selective 5-HT_{1B} receptor antagonist SB 216641 and the agonist CP 94253 to evaluate the role of 5-HT_{1B} receptors and their pharmacological stimulation in cocaine-maintained reinforcement and in drug- or the drug-associated cue-induced reinstatement. Additionally, the ability of 5-HT_{1B} receptor ligands to alter "natural" (food) reinforcement and relapse was examined. To this end, we used an intravenous cocaine self-administration model and an extinction/reinstatement task in self-administration [Przegaliński et al., Neuropeptides, 2005]. Moreover, we employed the food self-administration model using a procedure that paralleled closely cocaine self-administration.

Pretreatment with SB 216641 (2.5–7.5 mg/kg) did not alter the cocaine (0.5 mg/kg/infusion)-maintained responding nor did its highest dose change the dose-response relationship for cocaine self-administration (0.125–0.5 mg/kg/infusion). CP 94253 (2.5–7.5 mg/kg) given during cocaine maintenance sessions reduced the cocaine (0.5 mg/kg/infusion)-evoked reinforce-

ment and produced a downward shift in the dose-response curve for cocaine (0.125–0.5 mg/kg/infusion). No 5-HT_{1B} receptor ligand produced alterations in food-maintained behavior. Moreover, we observed that neither CP 94253 (5–7.5 mg/kg) nor SB 216641 (5–7.5 mg/kg) produced significant alterations in locomotor activity in non-habituated animals.

CP 94253 (5 mg/kg, but not 2.5 mg/kg) significantly attenuated the reinstatement of cocaine-seeking behaviors evoked by either the priming dose of cocaine (10 mg/kg, *ip*) or by the drug-associated cue while CP 94253 dose of 7.5 mg/kg (but not 2.5–5 mg/kg) reduced food-seeking behavior.

Our results indicate that 5-HT_{1B} receptors are not involved in cocaine reinforcement, while the pharmacological stimulation of these receptors facilitates cocaine-maintained responding and suppresses cocaine-seeking behaviors. The effects of the 5-HT_{1B} receptor agonist CP 94253 have been distinguished from the other goal-directed responding (lack of alterations in food-maintained behavior) and from changes in locomotor activity. These findings suggest that stimulation of 5-HT_{1B} receptors may be effective in modifying the abuse-related effects of cocaine and also show that such treatments may decrease the tendency to relapse.

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Interactions of serotonin (5-HT)$_{2c}$ receptor-targeting ligands and nicotine: locomotor activity and drug discrimination studies

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Nicotine is a drug of abuse acting *via* nicotinic acetylcholine receptors [Picciotto et al., Neuropsychopharmacology, 2000]. In animal models, nicotine has strong rewarding properties, serves as a discriminative cue and induces hyperlocomotion. Recently, it was found that some behavioral effects of nicotine could be influenced by manipulating the serotonergic (5-HT) system, mainly 5-HT_{2A} and 5-HT_{2C} receptors [Batman et al., Psychopharmacology, 2005]. In fact, it was shown that stimulation of 5-HT_{2C} receptors attenuated not only the nicotine-induced locomotor activity, but also reward [Grottick et al., Psychopharmacology, 2001].

The present study tested the hypothesis that 5-HT_{2C} receptors modulated the nicotine-evoked locomotor activity and the discriminative stimulus effects of nicotine in male Wistar rats. To this end, we used the selective 5-HT_{2C} receptor antagonist SB 242,084 (0.25–1 mg/kg, *ip*) and the 5-HT_{2C} receptor agonists Ro 60-0175 (0.3–3 mg/kg, *sc*) and WAY 163,909 (0.75–1.5 mg/kg, *ip*).

When given acutely to rats, nicotine (0.1–0.8 mg/kg) enhanced locomotor activity and the maximum effect was observed after 0.4 mg/kg. SB 242,084 (0.25–1 mg/kg) augmented the hyperactivity evoked by nicotine (0.4 mg/kg). Both Ro 60-0175 (0.3–3 mg/kg) and WAY 163,909 (0.75–1.5 mg/kg) given in combination with acute nicotine (0.4 mg/kg) decreased the locomotor response to that psychostimulant. In rats trained to discriminate nicotine (0.4 mg/kg, *sc*) from saline in a two-lever, water-reinforced fixed ratio 10 task, nicotine (0.05–0.4 mg/kg) evoked a dose-dependent substitution for the training drug. When tested alone, SB 242,084 (1 mg/kg) weakly substi-

tuted for the nicotine cue (31.82% drug-lever responding), while Ro 60-0175 (1 mg/kg) and WAY 163,909 (1.5 mg/kg) failed to generalize for nicotine (< 20% nicotine-lever responding). Given in combination with nicotine (0.05–0.4 mg/kg), SB 242,084 (0.5–1 mg/kg) did not alter the dose-response curve of nicotine, while Ro 60-0175 (1 mg/kg) and WAY 163,909 (1–1.5 mg/kg) dose-dependently attenuated the discriminative stimulus effects of nicotine.

The present data indicate that 5-HT_{2C} receptors play a crucial role in the locomotor and subjective effects of nicotine. Agonists of 5-HT_{2C} receptors may be considered as therapies for nicotine abuse.

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Expression of G-protein alpha-subunit mRNA at various periods after cessation of chronic morphine administration

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The phenomena of tolerance and dependence to psychoactive drugs are believed to involve both transient and long-term changes in gene expression in specific brain regions [Nestler et al., Science, 1997]. Morphine is a potent analgesic drug with known addictive properties. The identification of morphine-induced changes in the expression of genes is of critical importance for the understanding of addictive behavior. The heterotrimeric G-proteins play a critical role in brain signal transduction by coupling extracellular receptors to intracellular signaling pathways. G-proteins are composed of single alpha, beta and gamma subunits, they are widely distributed in the brain and can couple with a large family of G-protein-coupled receptors. Specific G-protein subtypes can activate different effectors and certain receptors can associate with more than one subtype of G-protein [Neves et al., Science, 2002].

In these studies, we have utilized a reverse transcriptase-polymerase chain reaction (RT-PCR) method to detect the levels of alpha subunits of four main G protein families: G(q/11), G(12), G(i/o) and G(s) in the frontal cortex, amygdala and nucleus accumbens of morphine-treated rats. Animals were injected with increasing doses of morphine (from 10 to 50 mg/kg *ip*, twice daily, 14 days) and were decapitated after 2 or 48 h from the last injection. Total RNA was isolated from the structures and reversely transcribed to cDNA and amplified in the PCR.

In the frontal cortex, no significant changes were observed 2 h after morphine administration, while during the withdrawal period the expression of Galpha(12) and Galpha(11) was significantly increased. In the nucleus accumbens, the expression of mRNA of all proteins was elevated during the withdrawal period, while the responses induced by morphine administration did not reach the level of significance. In the amygdala no changes reached the level of statistical significance. Two hours after the last morphine injection, the expression of Galpha(s-l) subunit was significantly elevated in the frontal cortex, and decreased in the nucleus accumbens, with no change in the amygdala. Galpha(i1) and Galpha(i2) mRNAs in the amygdala were depressed after chronic morphine treatment both shortly after the last morphine dose and after 48-h withdrawal. In contrast, the expression of Galpha(i2) was significantly elevated in the nucleus accumbens 2 h after the last dose of morphine, but normalized 48 h later. No changes were observed in the frontal cortex. The only significant change,

a marked increase in Galpha(oA) expression was observed in the nucleus accumbens, and only in the withdrawal period. No changes were observed in the frontal cortex and amygdala at either time.

In conclusion, we found that administration of morphine in the manner that made a rat morphine-dependent caused some structure-related changes in the expression of particular G proteins. Although elucidation of the functional significance of those changes requires further studies, the data demonstrate the involvement of various G proteins in the development of tolerance and/or dependence upon morphine.

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No cross-sensitization to the locomotor effects of morphine after repeated methadone administration in rats

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Repeated administration of psychoactive substances, including morphine, may cause, depending on the response studied, sensitization or tolerance to a later doses of the same or other psychoactive drugs. Sensitization allegedly plays an important role in the development of drug addiction. We were interested whether methadone can induce morphine-like locomotor activation and cross-sensitization to morphine.

Adult male Sprague-Dawley rats were given sc injections of physiological saline (1 ml/kg/day, 6 days/week, 14 doses), morphine sulfate (5 or 10 mg/kg/day, 6 days/week, 14 doses, or 10 mg/kg every other day, 7 doses), or methadone hydrochloride (1 mg/kg/day, 6 days/week, 14 doses, or 3 mg/kg every other day, 7 doses), followed by 14-day 'abstinence' period. After the break, all the rats were given a challenge dose of morphine sulfate (5 mg/kg, *sc*). Locomotor activity was assessed for 55 min after the 1st, 7th and 14th (in the rats injected 6 days/week) or the 1st, 4th and 7th injection (in the rats injected every other day), and after the challenge, beginning 30 min after the injections.

In drug-naive rats, the lower doses of morphine or methadone enhanced locomotor activity, whose activation was preceded by sedation in the morphine-, but not in the methadone-treated rats, whereas the higher doses of the drugs suppressed locomotor activity throughout the observation period. The repeated administration of morphine, but not of methadone, resulted in extinction of the sedation and enhancement of the locomotor activation except in the rats injected every other day. Compared to the saline-pretreated rats, those given morphine 6 days/week, but not those injected with morphine every other day or with methadone, showed increased locomotor activity after the morphine challenge.

Daily morphine administration causes lasting tolerance to its sedative and sensitization to its locomotion-enhancing properties. Despite some similarity in its acute effects, repeated administration of methadone does not cause cross-sensitization to morphine in the rat. This finding may be relevant to the utility of methadone in the substitution therapy of opioid addiction.

Morphine-conditioned reward and morphine-induced sensitization are inhibited by neuropeptide FF (NPFF)

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Emerging evidence indicates that pharmacological effects of morphine may be attenuated or suppressed by various endogenous peptides. One of these peptides is neuropeptide FF (NPFF). Recent data provide information that NPFF is involved in autonomic functions, memory and neuroendocrinological regulation, and also in pain mechanisms, and opioid tolerance and dependence. The pharmacological effects of NPFF result from its interactions with two receptors called: FF1 and FF2. They are widely distributed in the CNS and their localization is related to the distribution of the NPFF.

NPFF was first considered as an anti-opioid, but it is referred now to as an opioid-modulating peptide. The aim of the present study was to investigate whether NPFF influences the expression of morphine-induced sensitization to its hyperlocomotor effect and whether it modulates the rewarding effect of morphine, as measured in conditioned place preference (CPP) paradigm. The phenomenon of sensitization is involved in drug addiction, particularly in drug-seeking behavior. Rewarding effect of drugs induces pleasure and drug craving in humans and also plays a role in drug addiction.

Sensitization to hyperlocomotor effect of morphine was induced in mice by five repeated administrations of morphine (10mg/kg, *ip*) at 3-day intervals. Locomotor activity was measured in activity cages for 60 min. Seven days after the last morphine injection, NPFF (5, 10, 20 g, *icv*) was given immediately before the challenge dose of morphine (10 mg/kg *ip*).

Rewarding effect of morphine was measured in CPP paradigm in rats. CPP paradigm consists of three phases. In the first phase (pre-conditioning), rats were given free access to both (black and white) compartments of the apparatus for 15 min each day for 3 days. On day 3, the time spent by the animals in each compartment was recorded for 15 min. In the second phase (conditioning), which lasted 3 days, the animals received an injection of morphine (10 mg/kg, *ip*) before being confined to the drug-paired (white) compartment for 30 min. After a 4-h interval, rats received saline immediately before confinement in the vehicle-paired (black) compartment for 30 min. In the third phase (post-conditioning) the time spent by rats in each compartment was measured during 15 min of observation, after a single *icv* injection of NPFF at the doses of 5, 10, 20 g. The differences (in seconds) between post-conditioning time minus pre-conditioning time spent by rats in the drug-associated compartment were taken for statistical evaluation.

The acute administration of NPFF decreased the expression of morphine-induced sensitization. This effect was dose-dependent and statistically significant at the doses of 10 and 20 g. Furthermore, NPFF reduced morphine-induced CPP at the dose of 20 μg. The results of our study suggest an implication of NPFF in morphine dependence and in relapse to morphine addiction.

Effect of 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) on morphine mechanism of action in behavioral and neurochemical studies in rats

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Opiates are used clinically as analgesics, but their euphoric actions can lead to dependence and addiction. The activation of mu-opioid receptors by morphine produces the increase in dopamine release in the nucleus accumbens leading to activation of the reward system in this structure. 1MeTIQ is an endogenous substance with antidopaminergic properties [Antkiewicz-Michaluk et al., J Neurochem, 2001]. In our earlier studies, we have shown that 1MeTIQ strongly influenced morphine mechanism of action. It was demonstrated that 1MeTIQ potentiated morphine-induced analgesia as well as prevented the development of morphine tolerance and inhibited expression of abstinence syndrome [Wąsik et al., Eur Neuropsychopharmacol, 2005]. To continue these researches, we investigated the effect of 1MeTIQ on morphine-induced dopamine release in an *in vivo* microdialysis study, and, additionally, the influence of 1MeTIQ on morphine-induced hyperacivity was shown in behavioral experiments in rats. The concentration of dopamine (DA) and its metabolites (DOPAC, 3-MT, HVA) in synaptic cleft was measured using HPLC methodology.

Behavioral studies have shown that 1MeTIQ (50 mg/kg) completely antagonized hyperactivity produced by intraperitoneal morphine injection. *In vivo* microdialysis researches have shown that acute systemic administration of morphine (10 mg/kg) only slightly increased the DA release in rat striatum but the level of its metabolites: DOPAC, HVA and 3-MT was markedly elevated. Co-administration of 1MeTIQ with morphine resulted in a significant long-lasting increase in DA release in rat striatum. At the same time, the concentration of DA metabolites: DOPAC and HVA was significantly decreased, however, the concentration of an extraneuronal metabolite, 3-MT was dramatically elevated in mixed group.

The present results demonstrated clearly the dissociation between behavioral and neurochemical studies. In behavioral experiments, 1MeTIQ antagonized morphine-induced hyperactivity whereas in microdialysis study, it was shown that 1MeTIQ fortified morphine-induced dopamine release. The dramatic increase in 3-MT observed during co-adminstration of morphine with 1MeTIQ and the inhibitory effect of 3-MT on dopaminergic hyperactivity may explain these discrepancies.

Influence of brain dopaminergic system on cytochrome P450 activity in the liver

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The present study was aimed at investigating the influence of the lesion of brain dopaminergic pathways (tuberoinfundibular, nigrostriatal and mesolimbic) on the activity of cytochrome P450 (CYP) isoforms in rat liver.

The tuberoinfundibular pathway was damaged by injection of 6-hydroxydopamine (6-OH-DA; 150 mg/kg) into the rat tail vein. The animals were injected once or on two consecutive days, and were allowed to sur-

vive for 48 h or 7 days, respectively, after the first injection. The nigrostriatal pathway was damaged by stereotactic microinjection of 6-OH-DA (16 g) into the medial forebrain bundle, while the mesolimbic pathway was lesioned by administration of the same amount of 6-OH-DA into the ventral tegmental area (VTA). The animals were sacrificed 14 days after damaging the nigrostriatal and the mesolimbic pathways. To protect noradrenergic neurons, the lesioned animals were given intraperitoneal injection of desipramine (25 mg/kg) 30 min before 6-OH-DA. The lesion of those brain dopaminergic pathways was confirmed by determining the level of dopamine (DA) and its metabolites (DOPAC – 3,4-dihydroxyphenylacetic acid and HVA – homovanillic acid) in the median eminence (tuberoinfundibular pathway), nucleus accumbens (mesolimbic pathway) or substantia nigra and striatum (nigrostriatal pathway) using HPLC with electrochemical detection. The activity of CYP isoforms was assessed in the liver microsomes by measuring the rate of testosterone hydroxylation in positions 7 (CYP2A1/2), 16 (CYP2B1/2), 2 and 16 (CYP2C11), 2β and 6β (CYP3A1/2); caffeine 3-N-demethylation (CYP1A2); ethylmorphine *O*-deethylation (CYP2D) and warfarin 7-hydroxylation (CYP2C6). The amount of the metabolites formed *in vitro* was assayed using HPLC with UV or fluorometric detection.

Lesions of the dopaminergic pathways caused a marked decrease in the levels of DA, DOPAC and HVA in the median eminence, substantia nigra and striatum or nucleus accumbens (DA: to 20–31%; DOPAC: to 32–60%; HVA to 41–48% of the control values). At 48 h after the lesion of the tuberoinfundibular pathway, only the activity of CYP2B was significantly inhibited (to 56% of the control value). Seven days after the lesion of the latter dopaminergic pathway, statistically significant inhibition of CYP2B, CYP2C11 and CYP3A activities was observed (to 56–70% of the control). At the same time, the activity of CYP1A2 was considerably increased (up to 154% of the control). Fourteen days after bilateral microinjection of 6-OH-DA into the VTA, CYP3A activity was significantly reduced (to 69% of the control), while CYP1A2 activity was substantially elevated (up to 133% of the control). In contrast, the lesion of the nigrostriatal pathway did not affect any CYP isoforms.The obtained results indicate that the influence of brain dopaminergic system on CYP activity in the liver, observed in this study, is pathway- and isoform-dependent. It seems that dopamine by altering levels of pituitary hormones (tuberoinfundibular pathway) and/or cytokines (tuberoinfundibular and mesolimbic pathways) can affect CYP activity in the liver. Thus, dysfunction of the tuberoinfundibular and mesolimbic dopaminergic pathways (e.g. during long-term therapy with neuroleptics) may lead to significant changes in the activity of different forms of CYP. Such changes in the enzyme activity may be of physiological and pharmacological significance, since the tested CYP isoforms catalyze the metabolism of endogenous substances (e.g. steroids) and clinically important drugs (e.g. psychotropics, antibiotics, calcium channel antagonists).

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Role of cytochrome P450 (CYP) in dopamine formation

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The present study was aimed at determining which rat cytochrome P450 (CYP) isoforms (CYPs) were involved in the formation of dopamine from trace amines m- and p-tyramine. We also examined the dis-

tribution of the activity of isoforms of the CYP2D family in different structures of rat brain.

The experiment on tyramine hydroxylation to dopamine was carried out using cDNA-expressed rat CYPs (microsomes from baculovirus-infected insect cells expressing CYP1A2, 2A2, 2B1, 2C6, 2C11, 2C13, 2D1, 2D2, 2E1, 3A2). The amount of dopamine formed by individual enzymes was measured using the HPLC method with an electrochemical detection. Studies into the activity of CYP2D in the brain were performed on male Wistar rats using brain microsomes prepared from the following structures: nucleus accumbens, frontal cortex, substantia nigra, striatum, truncus cerebri, cerebellum and the remainder of the brain. The activity of CYP2D was assessed as a rate of the 1'-hydroxylation of bufuralol to 1'-OH-bufuralol. The amount of the metabolite formed *in vitro* was measured using the HPLC method with a fluorescence detection. Of the cDNA-expressed rat CYPs tested, CYP2D2 turned out to play a significant role in forming dopamine from both m- and p-tyramine. The activity of CYP2D varied throughout the brain. The ability of brain structures to catalyze bufuralol metabolism was as follows (pmol of 1'-OH-bufuralol/mg of protein/min): cerebellum (0.82) > substantia nigra (0.75) > truncus cerebri (0.61) > nucleus accumbens (0.53) > striatum (0.46) > frontal cortex (0.45) > the remainder (0.17).

The obtained results show the ability of rat CYP2D2 to convert tyramine to dopamine, the latter reaction being considered as an alternative pathway of dopamine production *in vivo*. Further studies need to be undertaken to determine whether the contribution of this pathway to the overall dopamine synthesis in the brain is of physiological importance. It is also necessary to examine significance of other isoforms of the CYP2D family in this process, especially CYP2D4 which is the main CYP2D isoform found in rat brain.

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Affinity of clozapine for dopamine D_1 and D_2 in the HEK 293 cells

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Dopamine D_1 and D_2 receptors have been long suggested to play a role in the pathophysiology and treatment of schizophrenia. Clozapine, one of the so-called atypical antipsychotic drugs, does not produce extrapyramidal syndrome or elevate prolactin levels in plasma. Despite many experimental approaches, the exact mechanism of action of this drug has not been fully elucidated so far. The D_1 and D_2 dopamine receptors are among several central neuroreceptors for which clozapine has been shown to display moderate affinity. Recent PET study with the use of a new D_1 receptor-selective ligand, [^{11}C]NNC112, have shown that receptor occupancy with clozapine in the primate brain was higher in the frontal cortex than in the striatum after administration of clinically relevant doses of the drug. One of the interpretations of these differences was that endogenous dopamine might have competed with radioligand binding. Similar arguments are being frequently provided while interpreting the binding of antipsychotic drugs, including clozapine to dopamine D_2 receptors.

Here we report the data concerning the affinity of clozapine for dopamine D_1 and D_2 receptors in the model system, devoid of endogenous dopamine. HEK 293 cells were transiently transfected with the plasmids pcDNA3.1 encoding human dopamine D_1 and

D_2 receptors, using calcium phosphate precipitation procedure. The cells were harvested 48 h after transfection. The radioligand binding parameters were estimated using the GraphPad Prism 2.0 curve-fitting program. Binding assays were performed with [^{3}H]SCH23390 (D_1 receptor antagonist) and [^{3}H]spiperone (D_2 receptor antagonist). For saturation analysis, radioligands were added at concentration ranging from 0.06 to 6 nM (for D_1) and 0.01 to 4 nM (for D_2). The density of the receptors (B_{max}) expressed in the HEK 293 cells was equal to 3.25 pmol/mg of protein for D_1 and 5.0 pmol/mg of protein for D_2 receptor. In competition analysis, clozapine was added at 10 concentrations between 10^{-12} to 10^{-4} M. The obtained results strongly suggest that there are two clozapine binding sites both at the D_1 and D_2 receptors. The estimated K_i were as follows: 2.9 and 131 nM for dopamine D_1 receptor; 0.2 and 632 nM for dopamine D_2 receptor. There were no statistically significant differences in these parameters if cells were transfected concomitantly with both D_1 and D_2 receptors. The values of low affinity site are very much similar to those obtained for clozapine in the rat brain tissue. However, the high affinity sites, observed in the present experiment might be important while discussing the mechanism of action of that drug.

Oxidative caffeine metabolism by recombinant human cytochrome P450 (CYP) isoforms

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Caffeine, one of the most widely and frequently ingested compounds throughout the word, is a useful enzymatic probe for its rapid and complete gastrointestinal absorption, its distribution throughout body water, and its low plasma binding, as well as for its short half-life, negligible first-pass metabolism, minimal renal elimination and biotransformation, the latter being virtually confined to the liver. 3-N-demethylation of caffeine to paraxanthine is used as a specific marker reaction for testing cytochrome P450 (CYP)1A2 activity in humans. However, caffeine is also oxidized in a few other positions of its structure, namely, apart from the 3-N-demethylation catalyzed by CYP1A2, the compound undergoes 1-N-demethylation, 7-N-demethylation and 8-hydroxylation. CYP isoforms capable of mediating the oxidative metabolism of caffeine were identified using recombinant human cytochrome P450 (CYP) isoforms. For each isoenzyme, the V_{max} and K_m values and the V_{max}/K_m ratio (intrinsic clearance) for each metabolic reaction of caffeine were calculated. On the basis of the rates of caffeine metabolism by individual isoforms and regarding the relative amount of each isoform in total CYP protein in human liver, the contribution of individual CYP isoforms (given as per cent) to the catalysis of the four metabolic pathways of caffeine was assessed.

At the therapeutic concentrations of caffeine (up to 100 μM), 3-N-demethylation to paraxanthine was the most specifically catalyzed by CYP1A2 (86%). 1-N-demethylation to theobromine was mainly catalyzed by CYP1A2 (77%), and to a lesser degree by CYP2C8/9 and CYP3A4 (6–7%). 7-N-demethylation to theophylline was catalyzed in a non-specific manner, though mainly by CYP1A2 (40%), and to a lesser degree by CYP3A4 (14%) and CYP2C8/9 (11 and 14%). Caffeine 8-hydroxylation to 1,3,7-trimethyluric acid was not specifically catalyzed, either, though preferably by CYP3A4 (31%) and CYP1A2 (29%), and to a lesser degree by CYP2E1 (11%) and CYP2C8/9 (8 and 9%). Interestingly, at a higher substrate concentration (1 mM), the contribution of CYP1A2 to the catalysis of the above metabolic pathway was considerably lower (17%), while that of CYP2C8/9 (15 and 14%) and CYP3A4 (35%) was higher. Similarly, the contribution of CYP1A2 to 1-N-

demethylation was reduced (from 77 to 68%), while that of CYP1A2 to 3-N-demethylation was enhanced (from 86 to 92%) at higher substrate concentrations.

The results of the present study indicate that CYP1A2 is the main CYP isoform responsible for caffeine metabolism (especially for the catalysis of 1-N- and 3-N-demethylation); furthermore, they point to a dose-dependent contribution of the isoforms studied to the oxidative metabolism of the substrate. Both 1-N- and 3-N-demethylation are specific reactions for testing the activity of CYP1A2 in humans *in vivo* and *in vitro*, while the 8-hydroxylation of caffeine may be useful for a preliminary simultaneous evaluation of changes in the activity of CYP3A4 (*in vivo*, by comparing it with CYP1A2 activity, or *in vitro* – at high concentrations of caffeine or during CYP1A2 inhibition).

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Cobalt chloride, a hypoxia-mimicking agent, disturbs receptor-mediated and does not affect directly driven stimulation of cAMP in rat astrocyte cultures

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Cerebral hypoxia is a common component of severe brain insults, including trauma and stroke. In brain cells, hypoxic (ischemic) conditions induce (pro)apoptotic events, which consequently lead to cell death. Cobalt chloride ($CoCl_2$) is widely used as a hypoxia-mimicking agent; it substitutes for the iron in the putative heme-containing oxygen sensor, which prevents or reduces oxygen binding and thus stimulates hypoxia.

The purpose of this study was to compare the synthesis of cAMP in [^{3}H]adenine-prelabeled rat astrocytes cultured under normoxic and hypoxic conditions and to see whether $CoCl_2$ affects cAMP production evoked by receptor-dependent drugs (pituitary adenylate cyclase-activating polypeptide, PACAP, vasoactive intestinal peptide, VIP, isoprenaline) and by a receptor-independent direct stimulator of adenylyl cyclase. PACAP and VIP are known to strongly stimulate cAMP formation acting *via* adenylyl cyclase-coupled receptors named VPAC1 and VPAC2 (displaying similar affinity for PACAP and VIP) and PAC1 (binding to receptors specific for PACAP). Diterpene forskolin is a powerful and widely used post-receptor direct activator of catalytic domain of adenylyl cyclase. Hypoxia was obtained by incubation of astrocyte cultures with 0.1 mM $CoCl_2$ for 24 hours.

The treatment of cultured astrocytes with PACAP (0.001–1000 nM), VIP (0.1–3000 nM), isoprenaline (10 μM) and forskolin (10 μM) resulted in a strong stimulation of cAMP production under normoxic (without $CoCl_2$) condition. However, in $CoCl_2$-treated glial cells the cAMP responses to PACAP, VIP and isoprenaline were significantly reduced, whereas that to forskolin was not, the latter showing similar effects under normoxic and hypoxic conditions.

In conclusion, the obtained results suggest that in cultured rat astrocytes, $CoCl_2$-induced hypoxia suppresses receptor-mediated activation of the adenylyl cyclase→cAMP pathway but does not affect non-receptor driven stimulation of cAMP production resulting from direct activation of adenylyl cyclase. It is suggested that hypoxia may somehow influence the interaction between receptors, Gs protein and adenylyl cyclase.

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Effects of prenatal dexamethasone on proliferation and maturation of cortical cells

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A synthetic glucocoticoid, dexamethasone (DEX) is used in pregnancies at risk of preterm delivery. The above treatment, however, has been shown to have several unwanted effects including development of the central nervous system. Thus, the present study was undertaken to investigate whether prenatal DEX influences neurogenesis and the fate of newborn neurons during the different stages of the rat cerebral cortex development. To achieve this aim, proliferation, migration and survival of cortical cells were investigated.

Pregnant rat dams were injected *ip* with DEX (0.1 mg/kg) from day 11 post conception till parturition. To examine the proliferation and migration of cortical cells, the incorporation of thymidine analog, the bromodeoxyuridine (BrdU) technique was applied. BrdU (50 mg/kg *ip*) was administered to pregnant dams at the time of origin of three cohorts of cortical cells, i.e. embryonic days (E) 12–13 (layer I, subplate), E 15–16 (cells destined to layers IV–VI) and E 18–19 (cells destined to layers II–III). In order to examine cell proliferation, embryos were removed 24 h after the second BrdU injection (E14, E17, E20). To study cell migration and survival, the brains of the offsprings on postanatal day 12 (P12) were used. BrdU-positive cells were detected by standard immunohistochemical procedure.

It has been found that DEX significantly decreased proliferation of all examined cohorts of cortical cells. The observed decrease reached 12.2% on E14, 20% on E17 and 13% on E20 (all differences were statistically significant, one-way ANOVA, $p < 0.05$). Further analysis of the distribution of cortical cells labeled during prenatal development indicates that DEX influenced laminar positioning of neurons in postnatal brain (P12) (two-way ANOVA, treatment *vs.* layer, $p < 0.05$). This result may suggest disturbances in cellular migration.

Moreover, in P12 brains, we observed an increase in the number of BrdU-labeled cells in cortical layers II–III (25%) and IV–VI (32%) of DEX-treated animals (two-way ANOVA, treatment *vs*. layer, $p < 0.05$).

Our findings indicate that prenatal low doses of DEX may influence the development and lamination of the cerebral cortex. Moreover, in spite of the impact on rate of proliferation, prenatal DEX may promote the survival of cortical neurons.

Further experiments aimed at analysis of developmental apoptosis may resolve the discrepancy between effect of dexamethasone on proliferation and migration.

Effects of PACAP, VIP and some related peptides on cAMP formation in rat neuronal cultures

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Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) are members of the structurally related superfamily of polypeptide hormones, which also includes peptide

histidine-isoleucine (PHI), peptide histidine-methionine (PHM), helodermin and secretin. PACAP and VIP are widely distributed both in the central nervous system and peripheral organs of various vertebrates and exert pleiotropic physiological functions. Both peptides exert their actions *via* common receptors: VPAC1 and VPAC2, which bind VIP and PACAP with similar affinity. Additionally, PACAP also stimulates PAC1 type receptors, which bind PACAP with much higher affinity than VIP. The adenylyl cyclase (AC)/cyclic AMP (cAMP) is the main intracellular signal transduction pathway coupled with all mentioned types of receptors.

The present work is a continuation of our previous studies on the effects of various neuropeptides, including PACAP and VIP, on the AC→cAMP-linked signaling pathway, carried out on slices of the rat cerebral cortex and rat primary glial cell (astrocyte) cultures. The aim of the present study was to answer the question to what extent neuronal cells contribute to the cAMP response to PACAP-VIP family of peptides observed in a functionally intact tissue represented by cerebral cortical slices. Thus, in the present work we have studied effects of two PACAP forms (short-27 and long-38 aa), mammalian and avian (chicken) forms of VIP (ie. mVIP and cVIP), porcine and human PHI (ie. pPHI and PHM), helodermin and secretin (all applied at a wide concentration range), as well as some non-peptide agents known for their ability to stimulate cAMP in different model systems, such as forskolin, isoprenaline and histamine (tested for comparative purposes) on cAMP response in primary [^{3}H]adenine-prelabeled neuronal cultures (originating from cerebral cortex of E16-E17 Wistar rat embryos). All forms of PACAP, VIP and PHI concntration (0.001–3 μM)-dependently and strongly stimulated cAMP production in neuronal cultures, displaying the following rank order of potency: PACAP38 > PACAP27 > mVIP ≥ cVIP ≥ PHI/PHM ≈ helodermin > secretin. Their effects on AC-driven signaling system in rat neuronal cell cultures were weaker than those in rat primary glial cell (actrocyte) cultures or in slices of the rat cerebral cortex. As expected, a β-adrenoceptor agonist isopreneline (10 μM) and a direct activator of adenylyl cyclase forskolin (10 μM) also strongly stimulated cAMP generation, whereas histamine (100 μM) had no effect.

In conclusion, the obtained results showed that the rat cerebral cortex-derived neurons were responsive to PACAP, VIP and some related peptides and possessed PAC1 and likely VPAC type receptors linked to the activation of AC→cAMP signaling system. Such responses indicate that neurons significantly contribute to the overall cAMP responses to PACAP-VIP family of peptides observed in intact brain tissue.

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Influence of CMX, an adenosine A_1 receptor antagonist, on the anticonvulsant action of newer antiepileptic drugs in maximal electroshock-induced seizures in mice

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Previously, it was reported that methylxanthines, non-selective antagonists of adenosine receptors, possessed proconvulsant activity in various experimental models of epilepsy. The aim of this study was to examine the influence of 8-cyclopentyl-1,3-di-methylxanthine (CMX, a selective adenosine A_1 receptor

antagonist) on the protective activity of newer antiepileptic drugs (AEDs; lamotrigine, topiramate and oxcarbazepine) in the maximal electroshock (MES)- induced seizures in mice. In addition, the effects of AEDs alone and in combination with CMX were tested on motor performance in the chimney test. Moreover, the effect of CMX on plasma concentrations of AEDs was evaluated.

The experiments were carried out on male Swiss mice weighing 20–25 g, housed under standard laboratory condition. All procedures have been approved by the Local Ethics Committee in Lublin.

CMX at a dose of 10 mg/kg decreased the threshold for electroconvulsions, being simultaneously, ineffective at lower doses. CMX (5 mg/kg) enhanced the anticonvulsant activity of lamotrigine and oxcarbazepine in the MES test, by reducing their ED_{50} values by 25 and 37%, respectively. In contrast, CMX did not affect the anticonvulsant effects of topiramate. Moreover, CMX (5 mg/kg) co-administered with AEDs at their ED_{50} values from the MES test did not impair motor performance of mice subjected to the chimney test. Noteworthy, CMX (5 mg/kg) significantly increased the free plasma concentrations of lamotrigine and oxcarbazepine, having no impact on those of topiramate.
The obtained results indicate clearly that CMX potentiated the protective action of lamotrigine and oxcarbazepine by elevating their free plasma concentrations in experimental animals.

Antiepileptic drugs influencing GABA transmission decrease synthesis of kynurenic acid *in vitro*

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Kynurenic acid (KYNA) is an endogenous tryptophan metabolite antagonizing the activity of ionotropic excitatory amino acid (EAA) receptors. Brain synthesis of KYNA occurs due to the activity of kynurenine aminotransferases (KAT I and KAT II).

KYNA was shown to display potent neuroprotective and anticonvulsive properties and its impaired production was implicated in the pathogenesis of epilepsy, neurodegenerative disorders and other pathologies.

In the present study, we have evaluated the influence of antiepileptic drugs tiagabine (TGB) and vigabatrin (VGA) on the synthesis of KYNA: a) *in vitro*, in rat cortical slices and b) in semipurified brain homogenate (KATs activities). KYNA was determined by HPLC and detected fluorometrically. In cortical slices, TGB at the concentration of 0.1; 0.5; 1; 3 and 5 mM decreased KYNA synthesis to 75% ($p < 0.01$); 67% ($p < 0.01$); 59% ($p < 0.001$); 60% ($p < 0.001$) and 57% ($p < 0.001$) of control, respectively. VGA at the concentration of 3 and 5 mM diminished KYNA synthesis to 72% ($p < 0.5$) and 52% ($p < 0.01$) of control, respectively. The activity of KAT I was decreased by TGB at the concentration of 0.1; 0.5; 1; 3 and 5 mM to 85% ($p < 0.5$); 60% ($p < 0.001$); 39% ($p < 0.001$); 31% ($p < 0.001$) and 30% ($p < 0.001$) of control, respectively. VGA used at the concentration of 0.1; 1, 3 and 5 mM diminished the activity of KAT I to 68% ($p < 0.001$), 53% ($p < 0.001$); 45% and 36% ($p < 0.001$) of control, respectively. The activity of KAT II was decreased by TGB at the concentration of 0.5; 1; 3 and 5 mM to 83% ($p < 0.5$); 52% ($p < 0.001$); 38% ($p < 0.001$) and 37% ($p < 0.001$) of control, respectively. VGA used at the concentration of 0.5; 1, 3 and 5 mM inhibited the activity of KAT II to 62% ($p < 0.001$); 24% ($p < 0.001$); 23% ($p < 0.001$) and 19% ($p < 0.001$) of control, respectively.

Our data suggest that TGB and VGA may reduce brain KYNA synthesis, what could, at least partially, contribute to the side-effects associated with their use.

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Chronic administration of bupropion affects the anticonvulsant activity of some conventional antiepileptic drugs in mice

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Bupropion hydrochloride (BUP, Zyban), an effective antidepressant, is recommended as first-line smoking cessation aid. After overdose or even at doses considered to be therapeutic, it may produce generalized seizures. Using maximal electroshock (MES) model of seizures in mice, we have recently demonstrated that BUP administered acutely has also anticonvulsant activity, with ED_{50} (effective $dose_{50}$, i.e. the dose protecting 50% of mice against convulsions) of 19.4 mg/kg.

The aim of the study was to determine, whether BUP injected intraperitoneally (*ip*) affects the protective activity and motor toxicity of four conventional antiepileptic drugs.

BUP at a single dose of 5, 7.5 and 10 mg/kg, but not at 12.5 and 15 mg/kg, did not influence the convulsive threshold in the MES test whereas the dose, not affecting the threshold during chronic administration (14 days; every 12 h) was reduced to 5 mg/kg. Chronic administration of BUP at the dose of 5 mg/kg did not change the protective activity of *ip* valproic acid and phenobarbital in the MES test. However, if co-administered with carbamazepine or diphenylhydantoin, it significantly enhanced their ED_{50} values from 13.4 to 16.5 and 9.9 to 13.2 mg/kg, respectively. BUP did not increase the liability of antiepileptic drugs to produce motor impairment measured in the rotarod test.

Our research shows that chronic BUP administration, despite promising reduction of convulsive threshold, does not affect or even in some cases inhibits the anticonvulsive activity of conventional antiepileptic drugs. The results argue against clinical combined use of BUP and conventional antiepileptics.

Spatial learning in selected lines of heavy and light mice

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The body weight is a simple measure of quantitative traits associated with multigene effect. In our study, we have investigated the correlation between body weight and spatial learning in selected lines of heavy and light mice. Mice have been selected at weaning for the low or high body weight for 94 generations.

To examine the behavioral differences in learning, memory, motor activity and motivation between the

selected lines, water maze test was performed. Water maze paradigm was carried out for six days by training mice using a four training trials per day. Spatial memory was assessed by a trial given 24 h after the last training trial. Motor activity and motivation were evaluated in the experiment with visible platform (cued task). At the time of experiment, mean body weight of the heavy line was 45.84 ± 0.74 g and in the light line it was 24.63 ± 0.81 g.

ANOVA for repeated measures showed significant differences in place learning between low and high body weight line [$F(2, 41) = 3.82$, $p < 0.04$]. Group with low body weight showed impairment of learning abilities (47.06 ± 1.18 s) compared to the high body weight line (36.17 ± 1.11 s). There were no significant differences between groups in the mean escape latency in cued task. However, we have seen lengthening of time of searching of the visible platform in group of mice with low body weight (47.60 ± 3.00 s) compared to the heavy line (34.92 ± 2.95 s) and the control (41.40 ± 2.69 s). These findings suggest improvement of abilities of learning of hidden platform position in the heavy line of mice. The presented results lead to the conclusion that selection of mice for high and low body weight over 94 generations resulted in significant differentiation in learning abilities.

New normalizing drugs: carbamazepine and clozapine – their influence on memory functions and anxiolytic effect in rats

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Carbamazepine (CBZ) is one of mood normalizing drugs that can be used in affective disorder episodes in humans. So far, it has not been possible to determine explicitly the effect of CBZ on cognitive functions. Clinical studies suggest that carbamazepine produces a lower risk of learning and memory deficits. In monotherapy it does not significantly impair the performance of cognitive tasks in epileptic patients. Clozapine (CLO) belongs to atypical antipsychotics with affinity for various dopamine receptor subtypes (D_1, D_2, D_4), 5-HT_{2A}, 5-HT_{2C} serotonin receptors and muscarinic subtypes. Neurological studies suggest that clozapine may improve cognitive functions in several aspects such as verbal fluency. No positive effect of clozapine therapy was observed, however, on operational memory in humans, although administering this drug in prolonged treatment of patients with schizophrenia was effective in improving the patterns of a number of cognitive functions.

The purpose of this study was to investigate the effects of carbamazepine and clozapine (in both single and chronic treatment) on memory and anxiolytic effect in rats.

CBZ was administered to male Wistar rats at a dose of 30 mg/kg over 60-min periods before the test. In chronic experiments, CBZ was administered to rats twice daily for 7 and 14 days. CLO was administered to male Wistar rats at the dose of 10 mg/kg over 30-min-periods before the test. In chronic experiments, clozapine was administered to rats for 7 and 14 days. Memory assessment was performed in Morris water maze test [Morris, Neurosci Methods, 1984] (the test is a standard model for both spatial learning and memory assessment). Anxiolytic effects were studied in a "two-compartment exploratory test" of Crawley [Crawley, Pharmacol Biochem Behav, 1981].

CBZ administered at a single dose (30 mg/kg) did not improve spatial memory, but in chronic treatment (after 7 and 14 days) the memory function was improved. CLO administered at a single dose (10 mg/kg)

did not improve spatial memory, but the memory function was improved after 7 and 14 days of treatment.

In the Crawley test, one parameter was increased after CBZ and CLO – the white square entries (WSE), showing that the rats were emboldened to move more freely in the white, lighted area.

In conclusion, it can be suggested that carbamazepine and clozapine have a memory enhancing effect after chronic treatment. This effect is probably caused by the influence of CBZ on the systems of brain neurotransmitters and of CLO on dopamine and serotonin receptors. The results seem to suggest a role for CBZ as a subsidiary agent in memory deficits, e.g. in bipolar affective illness, whereas CLO might be considered for use in therapy of schizophrenic patients, and both these agents would function as new mood normalizing drugs.

Examination of the effect of neuronal nitric oxide synthase blockade on rat's memory in the object recognition test

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The novel object recognition task in rodents is a non-spatial, nonaversive memory test which has been increasingly used as a powerful experimental tool in assessing drug effects on memory and investigating the neural mechanisms underlying learning and memory [Ennaceur et al., Behav Brain Res, 1988].

Our interest in this memory type is a consequence of wide-range investigations carried out by us and dedicated to the examination of the role of neuronal synthesis of nitric oxide (NO) in processes related to learning and memory, particularly, its participation in the influence of neuropeptides on these processes. However, studies conducted up until now have not yet clarified the role of neuronal nitric oxide synthase (nNOS) in that task. Therefore, the aim of the present research was to characterize the effects of systemic pharmacological blockade of nNOS on formation of object recognition memory in rats.

Experiments were performed on the adult male Wistar rats. Blockade of nNOS was evoked (immediately after the first trial in the testing session) by intraperitoneal injection of 7-nitroindazole (7-NI) as a relatively specific inhibitor of this isoform of NOS, at the dose of 30 mg/kg.

Performance of the object recognition task was tested in a 40 cm × 50 cm open field surrounded by 50 cm high walls, made of Plexiglas. All animals were given a habituation session where they were left to freely explore the open field for 3 min. No objects were placed in the box during the habituation trial. Twenty-four hours after the habituation, a testing session which comprised two trials was conducted. The duration of each trial was 3 min. During the first trial (T1), the apparatus contained two identical objects (A + A). After the first exploration period, the rat was put back in its home cage. Subsequently, after a delay of an 1-hour interval, the rat was put back in the apparatus for the second trial (T2), but now with two dissimilar objects, a familiar one (A) and a new one (B). The times spent on exploring each object during T1 and T2 were recorded manually.

Following Ennaceur and Delacour, the recognition index calculated for each animal was expressed by the ratio TB/(TA + TB) (TA = time spent on exploring the familiar object A; TB = time spent on exploring the novel object B).

The results obtained in the present study show that post-training nNOS blockade by systemic administration of 7-nitroindazole impaired formation of memory of a novel object recognition task in rats. These results may suggest that neuronal nitric oxide synthesis is required for the formation of object recognition memory.

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Antagonists of group I mGlu receptors influence MMP-2 and MMP-9 in rat hippocamus

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The antagonists of group I mGlu receptors are involved in processes of learning and memory [Car et al., Pol J Pharmacol, 2000, 2001, 2002; Conn et al., Annu Rev Pharmacol Toxicol, 1997; Nadlewska et al., Pol J Pharmacol, 2001, 2002, 2003; Riedel et al., Behav Brain Res, 2003; Schoepp et al., Trends Pharmacol Sci, 1993]. They regulate synaptic plasticity and intracellular factors responsible for possibility to acquire and consolidate a new information. Considerable evidence suggests an interrelationship among extracellular matrix reconfiguration, synaptic remodeling, plasticity, long-term potentiation and memory processes [Dzwonek et al., FEBS Lett, 2004; Kaczmarek et al., J EMBO, 2002; Szklarczyk et al., J Neurosci, 2002; Wright et al., Peptides, 2002]. Especially both acquisition and consolidation of memory are probably involved in this process.

The objective of this study was to determine whether hippocampal matrix metalloproteinases (MMP-2 and MMP-9) are involved in activity of antagonists of group I mGlu receptors. We used AIDA, MPEP and LY367385 to indicate which type of receptor is important in the proposed mechanism. Activity of MMPs was determined by gelatin zymography in homogenates from rat hippocampus.

Zymograms showed that MMP-2 (active and total forms), and proform and total of MMP-9 were elevated after administration of AIDA. No changes in the activity of MMP-9 but enhanced MMP-2 active and total forms were measured after injection of MPEP, a selective antagonist of mGluR5. There were significant MMP-9 (all forms) as well as MMP-2 (the active and the total activity) elevations after using LY367385, the antagonist of mGluR1.

In summary, we suggested the role of the antagonist of mGluR1 in reconfiguration of extracellular matrix in the hippocampus of rats produced by MMP-9 and MMP-2. Taken together, these data reveal new mechanism of AIDA and LY367385 activity in synaptic and behavioral plasticity.

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Role of mGluR1 and GABA in fear memory consolidation in the kindled rats

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Kindling is a validated model of epilepsy and epilepsy-related remodeling of neural circuits leading to alterations in behavior as well as neuromorphological, neurophysiological, and neurochemical processes. Synaptic plasticity, necessary for learning and memory (i.e. conditioning) is strongly modified by kindling as well as by group I mGluRs. In this study, potential differences in behavioral, biochemical (*in*

vivo microdialysis) and immunocytochemical (c-Fos) effects evoked by post-training intra-hippocampal injections of group I mGluR agonists and antagonists in kindled rats were examined. In the non-kindled group, AIDA (a mGluR1 antagonist) was found to increase while DHPG (a mGluR1–5 agonist) to decrease fear conditioning (a freezing reaction), examined 24 h after conditioning session. In the kindled group, freezing reaction was significantly attenuated after AIDA administration. In both kindled and non-kindled group, no effect of mGluR5 selective compounds CHPG (a mGluR5 agonist), and MPEP (a mGluR5 antagonist) was observed. *In vivo* microdialysis study was also performed to evaluate the influence of kindling and mGluR ligands on the levels of glutamate, GABA and aspartate in the hippocampus, during fear conditioning. It was shown that baseline concentration of GABA was lower, whereas aspartic acid and Glu/GABA ratio were higher in the kindled animals compared to the control group. Post-training assays revealed lower GABA levels in kindled animals receiving AIDA and decreased Glu in animals given AIDA, DHPG and CHPG. In the control group, the level of aspartate after DHPG was increased. In non-kindled animals after re-exposure to the aversive context during the testing session and 24 h after conditioning session, GABA concentration was decreased that was followed by an increased Glu/GABA ratio in animals which received AIDA, DHPG and CHPG immediately after training session. The levels of GABA, Glu and aspartate were decreased and the level of Glu was increased in kindled animals which received DHPG and CHPG, respectively. Cluster analysis followed by factor analysis of experimental data revealed an opposite pattern of changes between kindled or control animals according to which hippocampal GABAergic activity after training session as well as Glu/GABA ratio during testing session influenced the conditioned fear reaction. In the immunocytochemical study, the post-conditioning administration of AIDA in the kindled animals increased the c-Fos induction in DG layer of the hippocampus and basolateral amygdala, 2 h after exposure of animals to the conditioning session. In the control group, a decrease in c-Fos induction in basolateral amygdala in the post-training AIDA-treated group was observed. Classification Tree analysis showed that GABA level after training and c-FOS induction in basolateral amygdala and CA3 layer of the hippocampus during expression of fear-conditioned reaction had a comparatively large prediction accuracy in discriminating between kindled and control animals in these experiments. On the basis of these findings, it can be suggested that the enhanced neuronal activity evoked by kindling may be the result of an imbalance between the activity of central GABA and glutamate systems. The above-described changes evoked by kindling may lead to the conclusion about a crucial and opposite role of hippocampal mGluR1 and GABA (measured immediately after training session) in the consolidation of memory in kindled rats.

Effects of an inhibitor of serotonin and noradrenaline reuptake, sibutramine, on binge-type eating in rats

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The serotonin releasers and reuptake inhibitors attenuate appetite and decrease body weight [Halford et al., Pharmacol Biochem Behav, 1998]. For example, sibutramine hydrochloride (BTS 54 524; *N*-{1-[1-(4-chloro-phenyl)cyclobutyl]-3-methylbutyl}-N,N-dimethylamine hydrochloride monohydrate; Reductil; Me-

ridia), a potent serotonin and noradrenaline reuptake inhibitor *in vivo* [Buckett et al., Prog Neuropsychopharmacol Biol Psychiatry, 1988] is known to induce dose-dependent reduction of body weight in obese patients. Also preclinical studies in rats demonstrate that sibutramine dose-dependently inhibits food intake [Strack et al., Obes Res, 2002] and stimulates thermogenetic processes. The goal of the present study was to establish the model of binge-type eating in rats and to investigate the effects of sibutramine on this behavior.

The test was carried out on male Sprague-Dawley rats as described by Corwin et al. [Physiol Behav, 1998] with some modifications. Rats were kept individually in home cages with unlimited access to the standard laboratory diet and tap water, and were never food-deprived. One (control) group was offered a free access to the commercially available pork lard everyday for 2 h, starting 2 h prior to lights off. The second ("binged") group was offered a 2-h access to the lard on various days intermittently, so that the intervals between lard offers were never longer than 4 days. This procedure lasted for 3 weeks. After the binge pattern of eating has stabilized, the "control" and "binged" groups were further divided into the vehicle- and sibutramine (7.5 mg/kg)-treated. After two weeks of chronic administration, the drug treatment was discontinued but the parameters were monitored all the time for the next week, to investigate possible follow-up effects of active treatment.

Binge-type eating procedure did not increase daily total energy intake. During the 2-h sessions on the "binged" days, an increase in the lard intake compared to controls was noted, suggesting the development of binge-pattern lard consumption. During active treatment days, the daily total energy intake was decreased by sibutramine in "binged" and non-binged rats. The 2-h lard consumption was decreased by sibutramine in the "binged" group. During post-treatment days, binged and non-binged rats treated with sibutramine showed higher total daily energy intake and it seemed to be due to the increased daily lab chow intake. On post-sibutramine days, "binged" rats consumed the same amount of lard as vehicle-treated controls while non-binged rats decreased lard consumption. The present data suggest that sibutramine might be effective in the treatment of binge-type eating.

Influence of pethidine injection during premedication on visual evoked potential (VEP)

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Opioids are widely used for sedation and analgesia. Inhibition of numerous functions of the central nervous system, impaired perception and brain activity depend first of all on medication, dose and way in which it is administrated.

The aim of this study was to estimate the influence of low dose of μ opioid receptor agonist, pethidine on visual evoked potential (VEP) in humans.

Twenty patients (aged 31–79, mean 60, body mass 60–70 kg), were premedicated with Dolargan before eye surgery (15 for cataract, 3 glaucoma, 2 strabismus). The day before surgery patients signed informed written consent. VEP were induced according to ISCEV standards but using two active electrodes separately on the skin of left and right occipital regions. Only one eye (with better visual acuity) of every patient was selected for examination. In 9 patients with full visual acuity at least in one eye (5/5), transient-pattern VEP were induced. Stimulation of one check size 26' was used. In 11 patients before cataract surgery flash VEP were done. The study was performed in electrophysiological laboratory under permanent control of anesthesiologist. Two recordings were taken at the beginning. Then Dolargan (25 mg) was given intravenously. Next recordings were

taken after 5, 10 and 15 min. Directly after the last search, the patients were moved to the operation theatre. Amplitude and latency of positive waves (P_{100} of pattern VEP and P_2 of flash VEP) were calculated. T-test for paired data was used for statistical analysis.

Mean P_{100} latency was 118 ms before and 117 ms at 5, 10 and 15 min after Dolargan injection. The amplitude was 6.3 μV and 4.1, 5.4, 5.5, respectively. P_{100} amplitude was significantly lower 5 min after Dolargan injection only ($p < 0.05$). No differences between flash VEP amplitude and latency were found. In one female (66-year-old) the P_{100} amplitude after Dolargan injection decreased by more than 50%. This woman has taken for a few years benzodiazepins at night.
Premedication with Dolargan does not significantly change activity of visual system.

Influence of midazolam on visual evoked potentials (VEP)

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Midazolam, a derivate of benzodiazepine has proved to be very successful in reducing anxiety and stress pre-, peri-, and postoperatively with no significant effect on the vital signs of a healthy patient [Jerjes et al., Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2005]. Midazolam intravenous premedication is an effective way to reduce the frequency of postoperative nausea, and perhaps vomiting, and to increase patients' satisfaction [Bauer et al., J Clin Anesth, 2004]. It also induces temporary amnesia. Midazolam is effective for sedation during visual evoked potentials (VEP) examination of non-cooperative children [Pojda-Wilczek et al., Klin Oczna, 2002]. However, it is not clear if midazolam sedation may influence the VEP results. Recently, it has been found that midazolam premedication may affect audiological evaluation with stapedius reflex and transient evoked otoacoustic emissions tests and should not be used when it is necessary to examine patients under general anesthesia [Guven et al., J Laryngol Otol, 2006].

The aim of this study was to estimate the influence of midazolam on VEP.

Seven patients (aged 57–80, mean 69, weight 60–70 kg), were premedicated with Dormicum for cataract surgery. The day before surgery, patients signed informed written consent. Flash VEP were done according to ISCEV standards but using two active electrodes separately on left and right occipital regions. Only one eye of every patient was selected for examination. VEP examination was performed out of the operating theatre in electrophysiological laboratory under control of anesthesiologist. At the beginning, two stimulations were done. Then Dormicum 2.5 mg was given intravenously. Then, after 5 and 10 min flash VEP was registered. Amplitude and latency of positive wave P2 were calculated. T-test for paired data was used for statistical analysis.

Dormicum given intraveniously caused rapid sedation and somnolence of patients. One patient fell asleep after 5 min and the others were so sleepy that maximum time for observation was 10 min because the patients were not able to cooperate for longer time. Flash VEP latency was not significantly changed during the observation (mean P2 latency was 131 ms in the beginning, 135 ms, 138 ms after 5 and 10 min, respectively). P2 amplitude was significantly decreased ($p < 0.05$) after 5 and 10 min, in comparison with initial values (mean P2 amplitude was 8.6 μV at first, 5.5 μV and 6.0 μV after 5 and 10 min, respectively). Amplitude below 50% of initial values was observed 5 min after the injection in three patients older than 70 (73, 80, 80).

Midazolam given intravenously for premedication before surgery caused deep sedation (3–4 degree in Ramsey score) and decreased amplitude of flash VEP. Midazolam should be given carefully in older patients.

Photic control of pineal melatonin synthesis: an input from different photoreceptors?

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Biological rhythms (including diurnal/circadian, lunar, and annual cycles) constitute a common, fundamental feature of living systems. One of the most extensively studied circadian rhythms in vertebrates is that of melatonin (MEL), an indoleamine hormone produced by the pineal gland and retina. The biosynthesis of MEL occurs in a light-dependent rhythmic fashion controlled by the endogenous circadian clock, with high levels at night and low during the daytime [Zawilska et al., Pol J Pharmacol, 1999]. The rate of MEL formation is regulated primarily by serotonin *N*-acetyltransferase (arylalkylamine *N*-acetyltransferase, AANAT), the penultimate enzyme in the hormone biosynthetic pathway. Light is the dominant environmental factor controlling MEL synthesis, and as such it has two distinct effects on its production. Light at night acutely suppresses AANAT activity and MEL content. In addition, pulses of light appropriately timed reset the circadian oscillator generating the MEL rhythm in a phase dependent manner [Zawilska et al., Pol J Pharmacol, 1999]. We have recently demonstrated that MEL synthesis in the chicken pineal gland, in addition to being directly photosensitive, is acutely inhibited *in vivo* by retinally perceived light. This action was exerted by both full spectrum white light (WL), and by ultraviolet radiation (UV-A) [Zawilska et al., J Pineal Res, 2004; Rosiak et al., Neurosci Lett, 2004]. The aim of the present study was twofold: (1) to investigate whether WL and UV-A acting on the eyes only are capable of resetting the phase of the circadian MEL rhythm in the chicken pineal gland; and (2) to pharmacologically characterize the type of retinal receptor involved in this action of light.

AANAT activity in the chicken pineal gland fluctuated in a robust circadian rhythm. Exposure of chickens to WL or UV-A late in the subjective night caused a significant phase advance of the circadian rhythm of pineal AANAT activity compared to non-exposed controls. The phase-shifting effect of WL was markedly attenuated by pretreatment of birds with SCH 23390 (a selective D1-dopamine receptor antagonist), given intraocularly (*ioc*) into both eyes at the dose of 100 nmol/eye. SCH 23390 did not modify the action of UV-A. On the other hand, the UV-A-induced phase advance of the circadian AANAT rhythm was antagonized by MK-801 (a selective blocker of NMDA glutamate receptors; 3 nmol/eye). It is suggested that the regulation of pineal MEL synthesis in the chicken pineal gland by retinally perceived WL and UV-A might involve input from different retinal photoreceptors. The cascade of events triggered by WL and UV-A includes stimulation of retinal D1-dopamine and NMDA receptors, respectively.

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Dopamine and melatonin – two neuroregulators in the turkey retina

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Retinal adaptive physiology is mainly regulated by two established retinal neuroregulators: dopamine (DA) and melatonin (MEL) [e.g. Cahill et al., Progr Retinal Eye Res, 1995; Iuvone et al., Progr Retinal Eye Res, 2005]. DA, the major catecholamine of the vertebrate retina, promotes the light-adaptive physiology, and it is thought to be a "biochemical signal of light". In line with this, in retinas of several vertebrates light stimulates DA synthesis, release and metabolism. MEL, in turn, functions as a dark-adaptive paracrine substance. The release of MEL from photoreceptor cells occurs in a light-dependent rhythmic fashion controlled by an endogenous circadian clock, with high levels during the night. In retinas of pigeon, rabbit, chick, and duck, MEL acting on MT2 receptors has been shown to inhibit DA turnover and release [Adachi et al., Brain Res, 1998; Dubocovich, Nature, 1983; Nowak et al., J Neurochem, 1992; Zawilska et al., Neurosci Lett, 2003]. The aim of the study was to extend the current knowledge on MEL-DA interplay in the retina. In particular, we investigated the ability of the turkey retina to rhythmically produce DA and MEL. In addition, the influence of DA and MEL on the activity of each other was examined.

In the retina of turkey, steady-state levels of DA and DOPAC oscillated rhythmically throughout the day, reaching higher values during the day than at night. The observed rhythms were out of phase with daily oscillations in retinal MEL content and activity of serotonin N-acetyltransferase (AANAT; the penultimate and key regulatory enzyme in MEL biosynthetic pathway). Acute exposure of turkeys to white light at night significantly increased retinal levels of DA and DOPAC, and decreased AANAT activity and MEL content in the retina. Intraocular (*Ioc*) administration of quinpirole produced a dose-dependent decrease in the nighttime AANAT activity and melatonin in the retina. On the other hand, in the light-adapted turkeys, MEL given intraocularly significantly reduced the retinal levels of DA and DOPAC. These data provide evidence for the existence of a mutual negative interplay between DA and MEL in the turkey retina.

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Influence of the cannabinoid receptor antagonist SR-141716 on the voluntary ethanol intake in WHP rats

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The aim of the present study was to evaluate the influence of a cannabinoid CB_1 receptor antagonist, SR-141716, on spontaneous ethanol drinking in rats genetically selected for high alcohol intake and preference (Warsaw High Preferring-WHP rats). The single *ip* injection of SR-141716 at doses 2.5, 5.0 and 10 mg/kg significantly reduced ethanol intake. SR-141716 at doses of 5 mg/kg and 10 mg/kg diminished also food consumption.

These results indicate that CB_1 receptor antagonist SR-141716 is able to reduce spontaneous ethanol intake in WHP rats, but this effect is not specific as indicated by the decreasing effect of this compound on food consumption.