Oral presentations

Satellite Symposium

ALLERGOLOGY
CURRENT POSSIBILITIES IN THERAPY OF ALLERGIC DISEASES
– FROM GENE TO CLINICAL EFFICIENCY

Shifting the balance between anti-inflammatory pharmacotherapy with glucocorticoids and specific immunotherapy in asthma and allergic rhinitis

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Asthma and allergic rhinitis are Ig-E mediated diseases characterized by chronic airway inflammation. The key role in IgE-dependent allergic rhinitis and asthma management is played by methods inhibiting allergic inflammation: either in a nonspecific way as glucocorticoids (GCs) or in a specific way as specific immunotherapy (SIT). So far clinical practice has given priority to pharmacological methods with GCs, especially inhaled GCs (GCs-IH), because they powerfully suppress the early and late airway inflammatory response, improve the pulmonary function, reduce airway hyperreactivity and successfully control these diseases reducing their morbidity and mortality. In the widely accepted pharmacology-oriented therapeutic paradigm, SIT has been viewed as a second or third-line treatment. The so far minor importance of SIT in asthma and rhinitis treatment has been due to relatively insufficient knowledge of SIT’s mechanisms, and its remote and poorly documented therapeutic effects. Recent years have brought, however, new data about the mechanisms by means of which SIT evokes immunity to allergens. Numerous studies have confirmed the method’s high efficacy, additionally stressing its long-lasting effects persisting after cessation of the treatment and pointing out its preventive properties like: lesser occurrence of asthma in rhinitis patients who were administered SIT, and reduction in the occurrence of new allergisations. The fact that among the available method SIT is the only allergen-specific one, that is causal and specifically targeted at IgE-dependent diseases, makes us perceive SIT in asthma and rhinitis management not as a further-line but a complementary treatment. The growing importance of SIT also results from the recognized limitations of inhaled GCs’ therapy: the impossibility of the therapy’s permanent curative effect, adverse effects of GCs’ higher doses, unavailability of new drugs characterized by a potent local anti-inflammatory action, and limited perspectives of the method’s further dynamic development. Contrary to GCs pharmacotherapy, SIT is making a rapid progress, which is promising with regard to the prospects of its growing efficacy and safety; among SIT’s latest advanced strategies are: adjuvants such as monophosphoryl lipid A or nucleotide immunostimulatory sequences derived from bacteria that potentiate “antiallergic” Th1 responses, genetically modified non-IgE binding recombinant allergens, and allergen derived peptides. In view of the recent dynamic developments of SIT we can predict a continuation of the shit of balance from GCs therapy towards SIT.
The Sixteenth Day of Neuropsychopharmacology

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Redefinition of the role of the immune system in depression

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Earlier studies considered the immune system to be a victim of stress, depression and anything that activated hypothalamic-pituitary-adrenal axis and sympathetic nervous system. Effort of researchers was concentrated on showing how mental distress, often as a function of psychosocial stressors or major psychiatric illnesses, was associated with suppressed immune responses and led to an increased vulnerability to certain diseases, like infectious disease and cancer. Now the immune system is recognized to be an agent contributing to many pathologies inherent to mental illnesses. Regarding the molecular mechanism by which immune activation may contribute to behavioral alterations, proinflammatory cytokines were demonstrated to interact with multiple pathways relevant to depression including neurotransmitter metabolism, neuroendocrine function, synaptic plasticity and information processing. Proinflammatory cytokines stimulate the breakdown of tryptophan, the primary precursor of serotonin, into kynurenine and quinolinic acid, which exhibits neurotoxic properties. Proinflammatory cytokines also increase the activity and expression of the serotonin transporter so they reduce serotonin availability in the synapse. Cytokine signaling molecules alter glucocorticoid receptor function through direct glucocorticoid phosphorylation and/or protein-protein interactions. Cytokines block: 1) translocation of GR from the cytoplasm to the nucleus; 2) GR-DNA binding, 3) GR-mediated gene activity. Such effects of cytokine signaling pathways on GR function contribute to the development of glucocorticoid resistance, which may enhance inflammation and reduce glucocorticoid feedback inhibition. These advances may ultimately lead to the development of completely novel therapeutics for these devastating illness.

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Stress-related cognitive deficits: pathogenesis and some new treatments options

Emil Trofimiuk, Anna Walesiuk, Jan J. Braszko

The term “stress” refers to the physiological rearrangements that occur in response to novel or threatening stimuli. The maladaptive effects of repeated stress may include susceptibility to depression, panic disorder, posttraumatic stress disorder, drug abuse and cognitive impairment. The latter may occur alone or along with some other disorders triggered by stress or hypercortisolaeemia. The changes include also deluge of neuroendocrine events, most of them being associated with the hypothalamo-pituitary-adrenal (HPA) axis activation. Prolonged administration of the exogenous glucocorticoids (GCs) mimics a great majority but not all the effects of the excessive HPA axis activity which, mainly through GCs, causes number of the harmful neurochemical and neuroanatomical alterations in brain, mostly in hippocampus, amygdala and prefrontal cortex. As a result, neurogenesis is crippled, dendritic remodeling appears in the hippocampal areas CA1 and CA3 [McEwen, 2000] and the neuronal atrophy takes place [Sousa et al., 2000] leading ultimately to the decrease of its volume and reduction of total number of neurons and their ramifications [Arundine et al., 2003]. Analogous injurious alterations of the neuronal morphology take place in the medial prefrontal cortex (mPFC) [Radley et al., 2004] and in the medial amygdala [Bennur et al., 2007]. Also, the impairments embrace cerebral aminergic systems, chiefly the 5-HT1A receptors [Bowman et al., 2003], which are responsible for the control of neuronal excitability in hippocampus through inhibiting interneurons, thus causing intensified excitotoxicity of glutamate and death of neurons [Sarnyai et al., 2000, Arundine et al., 2003]. Moreover, a destructive impact of stress at the levels of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) [Belanoff et al., 2001] was described. DA, NE, 5-HT and GABA are implicated in cognitive processes partly by interaction with the cholinergic system, also involved in the deleterious effects of chronic stress on mental functioning. For example, the dopaminergic receptors in mPFC, striatum and hippocampus [Clinton et al., 2006] undergo considerable damage. Specifically, the changes in mPFC related to dopaminergic and noradrenergic transmission are responsible for the stressogenic disturbances of working memory.

The results obtained in our laboratory over the last 5 years concerning the use of the extracts of Hypericum perforatum and Ginkgo biloba for the prevention of these stress-related cognitive deficits in rats will be discussed in this presentation. Briefly, well defined impairments of the associative and visual spatial working as well as reference aspects of memory caused by the 2 h daily 21 day immobilization stress were prevented almost completely in the animals receiving either H. perforatum or G. biloba extracts. Interestingly, the extracts increased performance also in naive rats, though not in all tests. In a search for the participation of the GCs in the stress-related cognitive deficits we ran also groups of rats injected with the appropriate doses of corticosterone (stress hormone in rats) and found some differences with the stressed groups in their responses to the Ginkgo and Hypericum prevention.

Our results appear to open up a new possibility of safe therapeutic interventions in the increasingly important deleterious effects of stress on cognition.
It is generally accepted that endogenic neuropeptides [De Vied, Behav Brain Res, 1997] and neuronal nitric oxide synthase [Chapman et al., Neuroreport, 1992] can influence learning and memory processes. The physiological role of neuropeptides and neuronal nitric oxide synthase was confirmed both in the cellular mechanisms of memory (such as long-term potentiation and neuroplasticity) [Böhme et al., Proc Natl Acad Sci USA, 1993; Dubrovsky et al., Brain Res Bul, 2003; Wright et al., Prog Neurobiol, 2004] and in cognitive tests [De Vied, Life Sci, 1976; Estall et al., Pharmacol Biochem Behav, 1993; Gard, Eur J Pharmacol, 2002; Holy et al., J Physiol Pharmacol, 1992]. Moreover, our own studies have suggested the modulatory role of neuronal nitric oxide synthase in the beneficial effects of some neuropeptides on processes related to memory and learning [Holy and Wiśniewski, Pol J Pharmacol, 1994]. Estimation of the degree of the relationship between neuropeptides and neuronal nitric oxide synthase as well as its specificity within the range of types and formation stages of memory will be discussed in several aspects. Special attention will be devoted to behavioral tasks which demonstrate that neuronal nitric oxide synthesis is necessary to evoke the beneficial effects of angiotensin II and vasopressin on memory processes.

These results and data available in the literature suggest that the neuronal nitric oxide synthase/neuropeptide interaction may be considered to be an important factor in the regulation of memory.

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Interactions between the glutamatergic and GABAergic systems: implications of baclofen and antagonists group I mGluRs in hypoxia-induced disturbances

Halina Car

Glutamate and γ-aminobutyric acid are the major excitatory and inhibitory neurotransmitters in the mammalian central nervous system (CNS), and the coordination of these systems provides the network activity and synaptic neuroplasticity that are required for the complexity of the CNS functioning. Data reported in the literature indicate that there are interactions between these two systems: GABA is synthesized in GABAergic nerve terminals from glutamate; glutamate and GABA can regulate neurotransmitter release on the terminals of each other and, additionally, appear to be co-released with most other transmitters. The sum of inputs generating the excitatory or inhibitory postsynaptic potential by glutamate and GABA, respectively determines the propagation of information through neuronal networks. Both neurotransmitters are involved in long-term potentiation and long-term-depression as well as in learning and memory processes.

The glutamate and the GABA systems are considered to participate in a wide range of CNS disorders. Short-term hypoxia induces an imbalance between the glutamatergic and the GABAergic systems that is related to cognitive deficits. My own studies suggest...
that antagonists of group I metabotropic glutamate receptors and baclofen, an agonist of GABA<sub>B</sub> receptors, can cooperate in behavioral processes and influence hypoxia-induced disturbances. Partially, the observed effects are dependent on remodeling the extracellular matrix by metalloproteinases MMP-2 and MMP-9.

These results support the hypothesis that pharmacological manipulation of the excitatory and inhibitory neurotransmitter tone may protect neurons against a hypoxia-induced damage and the development of therapeutic strategies can be based on the glutamate and the GABA neurotransmitter systems.

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The effect of 1MeTIQ on morphine-induce changes in dopamine release: in vivo microdialysis studies

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1-Methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) is an endogenous substance present in the brain with antidopaminergic and recently demonstrated by us antiaddictive properties [Antkiewicz-Michaluk et al., 2001; 2005; Wąsik et al., 2005]: potentiated morphine-induced analgesia as well as inhibited expression of abstinence syndrome.

Continuing our research we investigated using in vivo microdialysis studies the effect of 1MeTIQ (50 mg/kg, ip) on dopamine release in rat striatum produced by acute and chronic administration of morphine (20 mg/kg, ip), and during morphine abstinence. Additionally, we checked in in vitro studies the affinity of 3-methoxytyramine (3-MT) to catecholaminergic receptors: dopamine D1 and D2 and noradrenergic α1 and α2 in rat brain structures. In vivo microdialysis studies have shown that both acute and chronic administration of morphine produced an increase of dopamine release in rat striatum. Co-administration of 1MeTIQ potentiated (about 350%, p < 0.01) an increase of DA release but in the same time much stronger fortified the increase (about 800%, p < 0.01) of 3-MT concentration in synaptic cleft. In morphine-abstinent rats co-administration of 1MeTIQ slightly increased of DA but strongly (about 300%, p < 0.01) the concentration of 3-MT. In vitro receptors studies have shown the affinity of 3-MT in nM concentration to dopamine and noradrenaline receptors.

The present result demonstrated for the first time in in vivo experiments that 1MeTIQ affected the morphine mechanism of action and shifted DA metabolism towards O-methylation resulted the strong increased of 3-MT in synaptic cleft. We postulate the important role of 3-MT in antiaddictive properties of 1MeTIQ.
Is the visual system in the brain receptor stories involved?

Stefan M. Pojda

Vision is an psycho/physiological process of whole brain, what at present can be demonstrated by Positron Emission Tomography. The activities at least in 32 and more places in various part of the brain during visual activity were found. Therefore vision is completely personal and depends from a lot of factors determining the normal or pathological function of the brain.

The Flash or Pattern Visual Electrophysiological Potential F/PVEP is a method by which we are able demonstrate objectively the visual activity of the brain. Actually, in the human clinic, we can even to give an approximate value of the visual acuity in malinger or in children mentally, physically or visually handicapped. The clinical method of FVEP was adapted to experimental use in rats to find any influence of some neurotransmitters and neuromodulators on brain’s visual activity. We have found that NE increased the N1 and P2, DA decreased P2 and 5-HT increased P2 of FVER. Then we become interested in the opiates and recently amphetamine, which can evoke visual hallucination. We have found that the shape and typical values of FVER after opiates and amphetamines are quite different. More heavy destruction of FVEP after opiates was found. But both opiates and amphetamine damaged P2, the peak which is responsible for the value of central visual acuity. Maybe, that shutdown perceiving the outside world through vision is a condition to have visual hallucinations.
Administration of PBN (N-tert-butyl-alfa-phenylnitrone) during status epilepticus reduces neuronal injury, improves cognitive functions but enhances epileptogenesis in immature rats

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To examine whether neuroprotective treatment during early status epilepticus (SE) can prevent or modify long-term functional outcome, free radical scavenger, N-tert-butyl-alfa-phenylnitrone (PBN), which was previously found to decrease neurodegeneration in immature rats was used in this study.

LiCl/pilocarpine SE induced in 12 (P12) and 25 days old (P25) rats was used to study effects of PBN treatment. PBN was administered in a total dose of 200 mg/kg (two times 100 mg/kg). Three months after SE, spatial memory and aggression were assessed using behavioral tests, spontaneous video/EEG was monitored for 5 consecutive days and then extension of the damage to temporal structures was evaluated using morphometry and stereological cell count.

PBN treatment did not affect SE outcome in P12 animals. In P25 group, learning abilities in MWM were improved in PBN-treated group compared to controls. In contrast, based on handling score PBN-treated animals were more aggressive. In addition, video/EEG monitoring demonstrated that PBN treatment during SE leads to increased frequency and duration of seizures in adulthood. Time spent seizing per 24 h was 9.3 times higher in PBN-group than in controls. Morphometric evaluation confirmed neuroprotective effects of PBN treatment in SE. Damage was significantly reduced in the hippocampus, perirhinal cortex and amygdaloid region. PBN-treatment also reduced loss of hilar neurons as assessed by stereology and partially prevented ventricle enlargement induced by early SE.

Our study demonstrated that long-term effects of PBN treatment are related to measured functional parameter and age at SE and that not all effects are positive.
What can we learn from the evidence on antiepileptic drugs teratogenicity?

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Antiepileptic drugs (AEDs) taken during pregnancy have long been associated with an increased risk of major congenital malformations (MCMs) and intrauterine growth delay. However, most of the studies, have been fraught with methodological shortcomings, and differences in ascertainment methods and classifications prevent meaningful data pooling. The risks for different AED regimes are difficult to define from published studies and are mostly unknown for those containing the newly licensed drugs. Most of published studies failed to explore other potential risk factors, like family history of malformations, genetic background. In the attempt to provide informations on the risks of MCMs for prenatal exposure to the ever increasing number of AEDs, pregnancy registries have been developed.

The results strongly point that monotherapy with the most commonly used AEDs is associated with an increase in risk of MCAs by two to three times, and that the magnitude of risk increases in offspring exposed to polytherapy. Available evidence suggest that maternal seizures do not increase the risk of MCMs, they may otherwise harm the mother and the fetus A clear-cut dose-dependent relationship between the extent of petal exposure and fetal outcome was found for valproate and recently for lamotrigine. Information about effects on fetuses of newer generation AEDs other than lamotrigine and oxcarbazepine is scant. Large scale studies may also clarify whether individual AEDs differ in their ability to cause specific anomalies. Finally, studies are urgently needed to investigate other potential adverse effects of AED exposure, with special reference to effects on postnatal intellectual development.

Hormones and seizure susceptibility

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Convincing evidence proved bidirectional relationship between hormones and seizure phenomena. In experimental studies, chemically-evoked convulsions significantly increased plasma levels of (ACTH), prolactin, vaso-pressin, glucagon, cortisol, and aldosterone. Electroconvulsions were associated with increases of brain (TRH) levels. Release of (CRH), vasopressin and oxytocin was enhanced after kindled seizures.

On the other hand, TRH, (GnRH) and somatostatin, exhibited anticonvulsant properties. In contrast, CRH proved proconvulsant action. Data on ACTH and vasopressin are contradictory and show their bidirectional effect on seizure susceptibility. Insulin-induced hypoglycaemia may trigger seizures. However, under normoglycemic conditions, assured by glucose administration, insulin was anticonvulsant. Cortisol and corticosterone increased, while deoxy-corticosterone and aldosterone decreased the seizure threshold. Numerous data demonstrate that triiodothyronine and thyroxin increase seizure susceptibility. Amongst sexual hormones, estrogens are generally considered as proconvulsant, while progestins as anticonvulsant hormones. However, there is also evidence on opposite effects of estrogens. Influence of testosterone on seizures depends on the metabolic route.
The hormone is mainly metabolized to 3α-androstanediol, a positive modulator of GABA<sub>A</sub> receptor. The second metabolite of testosterone is 17β-estradiol, a hormone with proconvulsant properties.

In humans, a number of reports suggest an antiepileptic role of TRH in intractable epilepsies. The antiseizure effect of ACTH in the treatment of West and Lennox-Gastaut syndromes was shown to be transient and age dependent. In men, testosterone reduced complex partial seizures. In women, catamenial seizures may be attenuated by GnRH agonists and progesterone, and exacerbated by estrogens. There were also two case reports on single tonic-clonic convulsions after an acute ingestion of levothyroxine.

**Sclerosis multiplex in epileptic patients**

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Multiple sclerosis (MS) is a chronic neurological disease with a wide spectrum of symptoms. The occurrence of epileptic seizures in patients with MS is relatively rare, but prevalence of epilepsy varies from 0.5 to 10.8%, and it is at least three to six times more frequent in MS patients than in general adult population.

This study was performed to analyze clinical characteristics of epilepsy in patients with multiple sclerosis and to examine the response to anticonvulsant therapy.

The study group comprised of 84 patients suffering from MS and hospitalized from January 2004 to December 2006 in Department of Neurology. The patients have been diagnosed for the co-existence of MS and epilepsy.

Out of 84 patients, 9 patients (5 men and 4 women) displaying seizure activity were selected. The seizures were partial with secondary generalization in 7 patients and 2 patients had more than one type of seizures. In all 9 cases anomalies in the EEG were found (periodic lateralized epileptiform discharges mostly in temporal region). Brain MRI showed scattered T2 hyperintensities in different localizations. All patients displaying seizure activity were treated with carbamazepine or valproate with good therapeutic responses.

The obtained data support earlier findings indicating that epilepsy occurs more frequently in patients with MS when compared to general population. It may also be concluded that seizure activity may be an initial symptom of MS and seizures are usually partial with secondary generalization. Finally, EEG-MRI-seizure type correlation can be observed in SM patients.

**Pharmacological analysis of postictal refractoriness after cortical epileptic seizures in rats**

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Overlasting activity of mechanisms responsible for arresting seizures is expressed as postictal depression. Postictal refractoriness – an inability to elicit seizure immediately after the termination of the preceding
Epileptic afterdischarges (ADs) were elicited by electrical stimulation of sensorimotor cortex in 25-day-old rats with implanted electrodes. The first stimulation always elicited AD, the second stimulation started one min after the end of the AD; then the drugs were intraperitoneally injected and the paired stimulation was repeated 10 min later. Drugs were selected to influence different neurotransmitter systems: GABA_\text{A} receptors – pentylenetetrazol, bicuculline, picROTOXIN, an inverse benzodiazepine agonist Ro 19-4603; GABA_\text{B} receptor antagonist CGP35348; agonists of glutamate receptors – NMDA, kainic acid; caffeine; naloxone. Doses were chosen on basis of our data on convulsant (and/or another) action of these drugs; each dose group was formed by 8–10 rats.

The first pair of stimulations demonstrated a failure of the second stimulation; it was not changed by any drug acting at GABA_\text{A} receptors as well as by agonists of glutamate receptors. In contrast, CGP35348, caffeine and naloxone were able to partially suppress refractoriness after cortical ADs – the second postdrug stimulation elicited an AD but it was shorter than the first AD in the pair.

Postictal depression after cortical epileptic ADs is not due to a single mechanism, at least three inhibitory systems play a role in this phenomenon.

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A role of neurotrophic factors in epileptogenesis

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Epileptogenesis can be regarded as a long-term anatomical and functional transformation of neuronal circuits, which ultimately leads to recurrent unprovoked seizures. Molecular mechanism of epileptogenesis has been only partially unraveled, however, an involvement of glutamatergic receptors, transcription factors, neuropeptides and neurotrophins in this process has been strongly postulated. In the present study, we estimated time-dependent changes in BDNF, HSP70 and proTRH gene expression in rat brain following pilocarpine-induced seizures, that is a well-established model of epileptogenesis [Turski, Pol J Pharmacol, 2000]. An in situ hybridization study showed a strong but transient increase in BDNF and HSP-70 mRNA level in several neocortical and hippocampal regions, and long-lasting (up to 7 days) induction of proTRH gene in dentate gyrus and piriform cortex.

Modern genomics requires high throughput technologies to monitor multiple gene expression simultaneously. cDNA arrays provide such an approach. A single experiment with the macroarrays can provide information about the expression of tens of genes at a time. We used GEAarray series macroarrays. Although the results are preliminary, they seem promising, since we were able to observe profound, time-dependent changes in the expression profile of genes coding for neurotrophic factors and their receptors in the rat brain several days after seizures i.e. during “silent” period of epileptogenesis, which precedes seizure occurrence.

Since pilocarpine-evoked seizures are associated with neuronal damage, the dynamic changes in gene expression may reflect regenerative processes, but also aberrant synaptogenesis and shaping of pathological neuronal circuits which promote epileptic discharges.
Antileukotriene drugs and NF-κB inhibitors as anti-atherogenic agents in apoE/LDLR – double knockout mice

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Sixty female 8-week-old apoE/LDLR – DKO mice (in each group n = 10), on mixed genetic background C57BL/6J'129/SvJ were fed by Western diet (consisting of 21% of fat by weight and 0.15% of cholesterol by weight). Experimental groups (in each n = 10) received the same diet, mixed with: MK-886 in a dose of 30 mg/kg/day, BAY × 1005 in a dose of 18.75 mg/kg/day, montelukast in a dose of 1.25 mg/kg/day, PDTC in a dose of 150 mg/kg/day and curcumin in a dose of 7.5 mg/kg/day.

At the age of 24 weeks these mice were sacrificed. Aortas differed in the degree of atherosclerosis between control group and experimental groups. Measured by “en face” method, the percentage of occupied by Sudan IV – stained surfaces were: 25.5 ± 2% in control group, whereas in MK-886-treated group 11.16 ± 0.7%, in BAY × 1005 group 15.16 ± 1.4%, in montelukast group 17.23 ± 1.8%, in PDTC group 15.63 ± 0.6%, and in curcumin group 19.2 ± 0.6%.

“Cross-section” of aortic roots revealed the difference in atherosclerotic lesions. Measured in 8 consecutive sections mean surfaces ± SEM, occupied by oil red – O stained changes were: 455494 ± 26477 µm² in control group vs. 263042 ± 20736 µm² in MK-886-treated group, 278107 ± 21824 µm² in BAY × 1005 group, 303599 ± 22735 µm² in curcumin group, 291695 ± 30384 µm² in PDTC group, and 299201 ± 20373 µm² in montelukast group.

To sum up, we described for the first time that in apoE/LDLR-double knockout mice inhibition of five lipoxgenase activating protein (FLAP) by MK-886 or BAYx1005, cysteinyl leukotrienes receptors by montelukast, as well as NF-κB by PDTC or curcumin inhibit atherogenesis.
Heme oxygenase and correction of endothelial dysfunction
Rafał Olszanecki, Jacek Jawień, Anna Gębska, Mariusz Gajda, Ryszard Korbut

Healthy endothelium prevents intravascular thrombus formation, regulates organ blood flow and inhibits inflammatory processes within vascular wall. Impairment of endothelium-dependent vasodilatation accompanied by prooxidant, proinflammatory, prothrombotic and proapoptotic switch in endothelial cell phenotype, is a hallmark of atherosclerosis, arterial hypertension, and vascular complications of diabetes and heart failure. Heme oxygenase (HO), especially inducible isoform HO-1, is the rate-limiting enzyme in heme catabolism and has been shown to play a vital role in protection against cellular stress. We demonstrate that induction of HO-1, using pharmacological inducers exerts antioxidant, anti-inflammatory and antiapoptotic effects and may prevent or reverse endothelial damage in vitro and in vivo, in various models of hypertension (2K1C, SHR) and atherosclerosis (ApoE/LDLR KO mice). It is tempting to speculate that endothelial induction of HO-1 using pharmacological or genetic approaches may represent valuable strategy of future treatment of cardiovascular diseases.

Pharmacotherapy of endothelial dysfunction in paod patients – own clinical investigations
Dorota Starzyk-Rażowska, Krzysztof Bieroń, Aleksandra Goszcz, Lilia Grodzińska, Ryszard Korbut

Progression of arteriosclerosis induces an imbalance between endothelial secretagogues leading to disturbances in maintenance of vascular tone, platelet aggregability and functioning of blood fibrinolytic system. It is believed that endothelium-dependent vasorelaxations that have been impaired by atherosclerosis could be reversed by L-arginine, molsidomine, and simvastatin. L-arginine is substrate for generation of NO by endothelial nitric oxide synthase-3 (NOS-3), molsidomine is direct NO-donor, acting via its active metabolite – SIN-1, while simvastatin affects function of vascular endothelium probably due to efficient up-regulation of NOS expression.

Our studies were carried out in patients with PAOD according to Fontaine stages IIa and IIb. The patients were treated with L-arginine for 1 month, or molsidomine for 1 month, or simvastatin for 3 months, in three separated studies.

Clinical estimations were performed in all trials before starting and after termination of the therapy. Laboratory tests were carried out on each examination day before and after drug administration and after termination of studies.

Pain free walking distance was elongated by the treatment with L-arginine, molsidomine and simvastatin. Total walking distance was elongated after L-arginine and simvastatin therapy. ABI was increased after L-arginine and molsidomine, however it remained unaffected by simvastatin therapy. The thresholds of proaggregatory concentrations of ADP and collagen were significantly increased by L-arginine, molsidomine and simvastatin. However, final improvement was seen only in simvastatin study. Fibrinolytic effect (shortening of ECLT) was observed for all studied compounds, but never the effect on fibrinolytic system was seen after termination of
Undesirable effects and negative interactions of drugs that modify function of vascular endothelium

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Drugs that modify endothelial function and thereby protect coronary and cerebral circulation against thrombotic incidents play an important role in pharmacotherapy of cardiovascular diseases. Unfortunately, effectiveness of these drugs can significantly diminished due to negative interactions with other drugs taken for coexisting diseases as well as by occurrence of adverse effects. Our investigations carried out at Regional Centre for Monitoring of Adverse Drug Reactions significantly indicate that negative drug interactions are the main cause of the worsening of the effectiveness of drugs used for pharmacotherapy of hypertension. The effectiveness of angiotensin converting enzyme inhibitors (ACEI), ATI-receptor blockers or β adrenergic drugs are greatly reduced in patients treated simultaneously with anti-inflammatory non-steroidal or/and sympathomimetic drugs. In turn, inhibitory effect of thienopiridyne on aggregation of blood platelets may be increased when combined with Ginkgo extract and non-steroidal anti-inflammatory drugs. Recently, findings on pharmacokinetic interactions with statins have gained practical importance. The result of such interactions can be worsening of statin hypolipemic effects on the one hand and increased toxicity in the form of myalgia and myotoxicity on the other. Crucial element which can lessen patient self discipline during prolonged use of medication can be the occurrence of side effects found difficult to cope with.
A series of experiments are proposed to demonstrate that serotonin 5-HT1A receptors are interesting target for novel antipsychotic drugs. In the first set of experiments we investigated the effect of ipsapirone on the dopamine outflow and its selectivity towards 5-HT1A receptors in the rat prefrontal cortex. Using a brain microdialysis method in freely moving animals, it was found that ipsapirone, 5 and 10 mg/kg dose-dependently enhanced the outflow of dopamine. The above effects of ipsapirone were mimicked by buspirone (2.5 and 5 mg/kg), another 5-HT1A receptor agonist. The effect of ipsapirone (10 mg/kg) on the dopamine outflow in the rat prefrontal cortex was antagonized by NAN-190, (1 mg/kg) and WAY 100135, (10 mg/kg), i.e. substances with agonistic/antagonistic and antagonistic properties in relation to 5-HT1A receptors. It is concluded that 5-HT1A receptor agonists may be involved in the regulation of dopaminergic neurotransmission in the rat cortex. Subsequently we evaluated the impact of conditioned stress on outflow of dopamine in the rat prefrontal cortex. Exposure of rats to an environment associated with aversive stimuli-foot shock enhanced outflow of dopamine in a similar way as seen during the conditioning session when foot shocks were applied. Ipsapirone (10 mg/kg, but not 2.5 mg/kg) and buspirone (2.5 mg/kg) enhanced basal outflow of dopamine. However when ipsapirone (10 mg/kg) and buspirone (2.5 mg/kg) were given to rats exposed to conditioned stress, the stress-evoked elevation in dopamine outflow was abolished. It is concluded that conditioned stress in vivo enhances dopaminergic neurotransmission in the rat prefrontal cortex, this effect being attenuated by ipsapirone and buspirone, which operate via serotonergic 5-HT1A receptors. It is concluded that dependently on the level of dopamine outflow agonist of 5-HT1A serotonin receptors may normalized the level of dopamine outflow in rat prefrontal cortex. Further arguments of involvement of 5-HT1A receptors in pathophysiology of schizophrenia are provided by experiments showing that drugs like MK-801 which imitates in experimental animals cognitive symptoms of schizophrenia evoked similar alterations in the density of above receptors as it was found in clinical studies on brains of schizophrenics. The binding of [3H]8-OH-DPAT to 5-HT1A serotonin receptors was increased after MK-801 (0.4 mg/kg) as was shown by autoradiographic studies in the frontal, cingulate and part of entorhinal cortex, subregions of the hippocampus and raphe nuclei. The above receptor changes were observed at 2 h and, in some brain regions, at 24 h after MK-801. In saturation binding studies, an increase in the Bmax value in the rat hippocampus was found after MK-801 (0.4 mg/kg) while no changes being noted in the Kd value. It is concluded that single administration of MK-801 may alter the density of serotonergic 5-HT1A receptors and in consequence influence the function of the central nervous as it was found in brains of schizophrenic subjects. We investigated whether the antagonist of 5-HT1A receptors, WAY 100135, was capable of modifying the psychostimulant and psychotomimetic effects of MK-801, a non-competitive antagonist of NMDA receptors. It was found that: 1) WAY 100135 (10 and 20 mg/kg, but not 1.25, 2.5, and 5 mg/kg) transiently, in a dose dependent manner, attenuated the locomotor stimulant effects of MK-801 (0.4 mg/kg). Given alone, WAY 100135 had no effect on the locomotor activity of rats; 2) WAY 100135 (1.25 and 2.5 mg/kg, but not 10 or 20 mg/kg), attenuated or abolished the disruptive effects of MK-801 on the sensorimotor gating measured in a prepulse-induced inhibition of the
acoustic startle response paradigm. WAY 100135 in all tested doses had no effect on the sensorimotor gating or amplitude of the acoustic startle response; 3) WAY 100135 (1.25, 2.5 mg/kg, but not 5 mg/kg) attenuated the detrimental effects of MK-801 on working memory and selective attention, measured in a delayed alternation task. Again, given alone, WAY 100135 did not influence the behavior of rats in that experimental paradigm; and 4) MK-801 (0.4 mg/kg) had no effect on the 5-HT1A receptor mRNA level in rat hippocampus, measured 2 and 24 hours after MK-801 administration. These data indicate that 5-HT1A receptors might be involved in the psychotomimetic effects of non-competitive NMDA receptor antagonists. In addition, 5-HT1A serotonin receptor antagonists and partial agonists may have potential antipsychotic properties. The final study was designed to investigate the distribution of serotonin 5-HT1A receptor protein (5-HT1A-immunoreactivity) and its localization within cortical pyramidal neurons of the rat medial prefrontal cortex. This experimental direction was again inspired by data showing the role of 5-HT1A receptors in the pathology of schizophrenia. It was found that 5-HT1A-immunoreactivity was densely distributed in neuronal eyelash-like elements, and their size, shape and spatial orientation may suggest concentration of 5-HT1A-immunopositive material in the proximal fragments of axons of cortical neurons. Moreover, it was observed that these 5-HT1A-immunopositive fragments were present predominantly on proximal fragments of axons of pyramidal neurons, which was evidenced by double labeling experiments using glutamate and non-phosphorylated neurofilament H as markers of the cortical pyramidal cells. The 5-HT1A receptor immunoreactivity was localized distally to the inhibitory GABA-ergic terminals of chandelier and basket cells surrounding the pyramidal cell bodies and occasionally surrounding short initial segment of axonal hillock of pyramidal neurons. These anatomical data indicate that 5-HT1A receptors might control the excitability and propagation of information transmitted by the pyramidal cells. In general our results indicate that drugs operating via 5-HT1A receptors in the prefrontal cortex may constitute an important target for drugs used to repair dysfunction of neurotransmission in prefrontal cortex which is observed for example in schizophrenia and offers a new important target for novel neuroleptic drugs.
Influence of antidepressant treatment on the expression and function of group III metabotropic glutamate receptors in the rat brain

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Earlier studies showed that chronic electroconvulsive shock (ECS) or imipramine treatment induced a sub-sensitivity of group I metabotropic glutamate receptors (mGluRs) in hippocampus as well as the receptor protein level was increased in this structure. In this study the effect of chronic imipramine (10 mg/kg, 21 days) or citalopram (10 mg/kg, 21 days) treatment on mGluR4 and mGluR7 protein level in the cortex and hippocampus was examined using Western blotting procedure. In the hippocampal or cortical slices we examined also the influence of antidepressant drug administration on forskolin-stimulated cAMP formation. Non-selective agonist of all receptors belonging to the III group of mGluRs, ACPT-1, was used to establish their effects on the stimulation of cAMP production by forskolin.

It was found that mGluR7a-immunoreactivity was decreased after citalopram, but not imipramine treatment both in the hippocampus and in the cortex. No changes were observed in the mGluR4a-immunoreactivity. Prolonged treatment with antidepressant drugs failed to change the action of group III mGluRs agonist, ACPT-1, on forskolin-stimulated cAMP accumulation. Our results suggest that mGluR7, but not mGlu4 receptor is engaged in the action of an antidepressant drug, citalopram, in the brain regions which are considered to be implicated in the clinical response to antidepressant therapy.
Antidepressant activity of mGluR5 antagonists

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Involvement of glutamate system is demonstrated in the pathophysiology and treatment of depression. Ionotropic and metabotropic glutamate receptors participate in the mechanism of antidepressant action.

mGlu5 receptor antagonists demonstrate antidepressant activity in preclinical tests. MPEP, MTEP, AIDA are active in forced swim and tail suspension tests or olfactory bulbectomy animal model of depression. Also, zinc, non-specific antagonist of mGluR5 and NMDA receptors, exhibits antidepressant activity in forced swim/tail suspension tests, and in animal models of depression (olfactory bulbectomy, chronic unpredictable stress, chronic mild stress). Chronic treatment with MPEP, MTEP or zinc induced adaptation in BDNF gene expression and serotonergic (5HT2A and 5HT1A) receptors, similar to that changes induced by most antidepressant treatments.

All the preclinical data indicate that antagonism of mGlu5 receptors induce antidepressant effect.

Neuroprotective potential of mGlu1/mGlu5 receptor antagonists

in vitro and in vivo

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The aim of these studies was to evaluate neuroprotective ability of the antagonists of GI mGluRs, mGlu1 and mGlu5, in various in vitro and in vivo models of excitotoxicity and brain ischemia. There are conflicting literature data in regards to effectiveness of these ligands.

In the in vitro experiments we used primary cultures of rat cerebellar granule cells challenged with acute and sub-chronic D,L-homocysteine toxicity. Our data demonstrated that antagonists of the NMDA receptors (MK-801, memantine and amantadine), as well as mGlu1 and mGlu5 receptor antagonists (LY367385 and MPEP) given separately have limited neuroprotective potential, whereas simultaneous application of the NMDA receptor antagonists and antagonists of both subtypes of GI mGluRs provided almost complete neuroprotection. In vivo experiments using a gerbil model of global forebrain ischemia and a model of perinatal hypoxia (hypoxia-ischemia of 7-day-old rats) revealed that post-treatment with mGlu1 receptor antagonist EMQMCM induced neuroprotection in a dose-dependent manner in both models, while mGlu5 receptor antagonist MTEP was neuroprotective only in gerbils. Reduction of the spontaneous postischemic hyperthermia by EMQMCM, may be partially involved in the mechanism of neuroprotection. Moreover experiments in gerbils demonstrated that both GI mGluRs antagonists do not interfere with induction of ischemic tolerance by preconditioning ischemia.

These results demonstrate that the antagonists of mGlu1 receptors provide neuroprotection in various experimental models, although the mechanism of this action may be complex, while mGlu5 receptor antagonism induces no neuroprotection in the immature rat brain.

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A dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis activity, manifested as hypersecretion of glucocorticoids or disturbance in glucocorticoid receptor (GR) action, is often observed in major depression and schizophrenia. Antidepressants, antipsychotics and neurosteroids are known to ameliorate some deleterious effect of glucocorticoids on brain function, however it is not clear whether these agents can directly affect the functional activity of GR.

To answer this question, we investigated the effects of some neurosteroids, antidepressants and antipsychotics on corticosterone-induced gene transcription in mouse fibroblast cells (L929), stably transfected with mouse mammary tumor virus-chloramphenicol acetyltransferase (MMTV-CAT) plasmid (LMCAT cells). We found that allopregnanolone (1–100 µM) lesser extent, its isomers (5b-pregnan-3a-ol-20-one and 5a-pregnan-3b-ol-20-one), but not dehydroepiandrosterone sulfate, inhibited the GR-mediated gene transcription. Among antidepressant drugs under study, the most potent inhibitory effect on GR function was exerted by imipramine, amitriptyline, desipramine, fluoxetine and mianserin, while new generation drugs such as reboxetine, venlafaxine and mirtazapine had a weak effect on this parameter. On the other hand, of 6 antipsychotic drugs studied, only chlorpromazine and clozapine had profound inhibitory effects on GR-induced gene transcription. Furthermore, the combined treatment with allopregnanolone and antipsychotic drugs (chlorpromazine and clozapine) had a moderate, additive inhibitory effect on GR-induced gene transcription, whereas allopregnanolone did not enhance the effect of an antidepressant imipramine. Regarding the mechanism of allopregnanolone action on GR function, we found that this neurosteroid inhibited protein kinase C (PKC) activity, decreased the level of PKCa isoenzyme in the membrane fraction and decreased the amount of active phosphorylated form of extracellular signal-regulated kinase – mitogen-activated protein kinase (ERK-MAPK), which is the main kinase responsible for GR phosphorylation and regulation of its function.

The allopregnanolone-evoked attenuation of GR function, most likely connected with inhibition of PKC and ERK-MAPK pathways, resembles the effects of antidepressants and antipsychotics pointing to a common mechanism by which these agents ameliorate glucocorticoids action in manic-depressive disorders. Furthermore, allopregnanolone enhanced anti-glucocorticoid action of chlorpromazine and clozapine, but not that of imipramine, which suggests a potential clinical usefulness of a joint treatment with the neurosteroid and some antipsychotics.
Contemporary concept of placebo usage in clinical drug trials

Krystyna Orzechowska-Juzwenko

The placebo-controlled trials have been almost always controversial since the beginning of introduction of this method into new therapy investigations. From the legal and ethical point of view the usage of placebo is more and more frequently criticized because it deoids the patients of standard therapy advantages. However, recently the debate has become polarized. One view-called “placebo orthodoxy” is: that methodological considerations make placebo-controlled trials necessary unless there is an increased risk of death or irreversible morbidity associated with its use. The other view—which might be called “active control orthodoxy”, is: that placebo orthodoxy sacrifices ethics, the rights and welfare of patients to presumed scientific rigor; and if an effective therapy exist, the use of placebo should be prohibited. The third view – represents a middle ground view, in which the placebo-controlled trials are permitted, but only when the methodologic reasons for their use are compelling, a strict ethical evaluation has made it clear that patients who receive placebo will not be subject to a serious harm, and provisions have been made to minimize the risk associated with the receipt of placebo. Therefore, contemporary tendency to solve of ethical placebo problem connected with clinical trials of new drugs is often – application of more safe methods of results objectivization in new therapy by comparison of the results of clinical trials in the group taking a new drug to be tested to the one treated by conventional, standard, established the best so far known, pharmacotherapy.

Pharmacogenetics as a tool for individualized drug therapy

Marek Drożdżik

Pharmacogenetics blends important components of the disciplines of genetics and pharmacology, and aims to describe the influence of inheritance on variable drug response. Clinical observations of inherited differences in drug effects were first documented in the 1950s, and were related to variability of drug me-
Tabolizing enzymes’ activities. The discovery of the first pharmacogenetic molecular defect associated with polymorphic forms of CYP2D6 underlying altered response to debrisoquine was reported in the late 1970s. So, the field of pharmacogenetics began with a focus on drug metabolism, but it has been extended to encompass the full spectrum of drug disposition, including a growing list of transporters that influence drug absorption, distribution, and excretion as well as drug receptors. There are now numerous examples of associations between drug target polymorphisms and drug effects, which are crucial for clinical practice. Important example is the intolerance to azathioprine and 6-marcaptopurine due to thiopurine methyltransferase (TPMT) deficiency, determined by inheritance of TPMT mutated allele. Extreme intolerance to the drugs involves severe and even fatal cases of bone marrow aplasia. Another example of clinically relevant drug metabolizing enzymes’ polymorphisms affect CYP2D6, CYP2C9 and CYP2C19. Polymorphic forms of the allele modify response to antidepressant drugs and opioids (CYP2D6), warfarin (CYP2C9) and efficacy of Helicobacter pylori eradication (CYP2C19 is implicated in omeprazole metabolism). Genetically determined structure of receptors or other drug targets can also modify drug response. The best studied examples involve β2-adrenergic receptors (treatment of asthma with β2-adrenomimetics), dopamine receptors (neuroleptics) or ion channels (associated with long QT syndrome). Altered function of drug transporters, mediating drug absorption, distribution and elimination may be also important predictors of drug response, i.e. polymorphism of P-glycoprotein (encoded by MDR1 gene) involved in transport of immunosuppressants, anticaner drugs, glucocorticoids, protease inhibitors, statins as well as serotonin transporters (antidepressants). Based on the current knowledge it seems reasonably to state that pharmacogenetics has the potential to significantly enhance the ability of clinicians to use medications in a safe and effective manner, as such, represents an exciting field with tremendous clinical potential.

**Proinflammatory cytokines in atherosclerotic patients**

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The endothelial injury theory which leads to vessel myocytes proliferation and migration was recommended in 1973 by Glomset and Ross. The major injurious factors that promote atherogenesis, as smoking, hyperlipidaemia, hyperglycaemia are well known. A lot of proinflammatory molecules experimental were recognized in studies as well as in atherosclerotic patients. That is why from a pathological point of view all stages of atherosclerosis might be considered as an inflammatory response to injury. It is in agreement with well-accepted theory described as reaction to injury. Connections between the concentration of proinflammatory cytokines, chemokines and serum lipoproteins are under present investigations. There are also attempts of comparing the amount of above mentioned molecules with atherosclerotic plaque dimentions and increased artery wall thickness. Much more promising seems to be describing the role of inflammatory cells’ products in vascular risk stratification. The role of multicenter clinical studies is lower because of their hand endpoints and methodological limitations. Restrictive inclusion criteria requiring accurate diagnosis of inflammation raise doubts. For some investigators it is only preselection, which reduce the real value of achieved results. Antinflammatory and vasoprotective influence of hypolipemic and hypotensive drugs is considered as an important clinical supplementation to their basic mechanism of action. It could not be ruled out that this additional effects of drugs is responsible for better outcomes in the treated patients. Generally, precise dividing between the mechanisms of drugs actions is usually impossible in clinical trials conditions. Although we can measure different molecules, hs – CRP assay represents the best choice at this time.
Clinical value of detection of serum neopterin in patients with chronic heart failure

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Current research indicates that neopterin might arise as a potential marker of cardiovascular disease. Elevated neopterin is present in conditions associated with increased activity of monocytes/macrophages, including chronic heart failure.

Aim of the study was evaluation of clinical value of neopterin assessment in patients with chronic heart failure.

Serum neopterin assessment was conducted in 68 patients with heart failure (CHF) NYHA class II–IV. Control group consisted of healthy volunteers. Neopterin was assessed by radioimmunoassay (RIA) with MP Biomedicals. In patients with CHF, statistically significant elevation of neopterin concentration was observed compared with control group. There was a correlation between disease stage and neopterin elevation.

In patients with CHF, there is a marked elevation of serum neopterin levels corresponding to heart failure progression.

Increased concentrations of neopterin in patients with CHF can be used as a marker allowing for therapy control and a prognostic tool evaluating CHF progression.

Investigation of MDR polymorphisms as a risk factor of cancer

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The best-studied drugs and xenobiotics transporter is P-glycoprotein (P-gp) encoded by the Multi-Drug-Resistance (MDR1) gene was initially described in cancer pharmacotherapy as responsible for the occurrence of multidrug resistance. Further investigations indicated physiological role of P-gp, which generates the ATP-dependent cellular transport and acts as an efflux pump providing a barrier against the entry of toxic compounds into the cells and removing xenobiotics after they have entered. After identification of many drugs that are P-gp substrates the role of this protein for carcinogens accumulation was postulated.

The role of MDR1 polymorphism in carcinogenesis is not clear. The MDR1 could increase the transport of toxic xenobiotics from cells and therefore protect them from the death. Otherwise, some data showed the role of MDR1 in cell differentiation and apoptotic processes. It was suggested that one way of deactivation of cell death pathway is stimulation of responsive element in the MDR1 promoter through β-catenin complex. It was shown that in early stages of different cancer the level of β-catenin is accumulated and then probably interaction between MDR1 responsive elements and β-catenin provides growth and survival advantages for epithelium cells. MDR1 was recognised as a target gene of the T-cell/lymphoid enhancer factor (TCF4)/β-catenin complex, which stimulates responsive element in the MDR1 promoter.

In combination with cell proliferative activities of c-myc and cyclin D1, MDR1 may initiate tumorigenesis by suppressing cell death pathways. Furthermore, P-gp inhibits caspase-8 activation and in this way also regulates Fas induced apoptosis.

Recently the association between an MDR1 polymorphism C3435T in exon 26 and susceptibility to renal epithelial, colorectal, endometrial, haematological malignancies, and glioblastoma was shown. Current studies enlarged the view on the impact of genetically determined differences of P-gp activity on carcinogenesis and supported hypothesis that MDR1 polymorphism could be a useful biomarker showing susceptibility to develop cancer.
Influence of magnetic field on hemodynamic and biochemical parameters of perfused isolated rat’s heart subjected to ischemia

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An inflammatory response of cardiomyocytes subjected to ischemia followed by reperfusion mediated by cytokines is one of the postulated mechanisms that trigger the ischemia-reperfusion injury of the heart. In the following study the potential beneficial cytoprotective effect of magnetic field (MF) on ischemic heart was estimated.

Isolated rat hearts perfused with Krebs-Hanseleit’s solution were subjected to ischemia for 20 min. The study group (n = 6) was treated with MF (220 µT) for 8 min during the ischemic period. After the ischemia 30 min reperfusion time was given. Two control groups (ischemia without magnetic field, n = 6 and a group perfused without ischemia, n = 6) were established.

Left ventricular function measured by RPP (rate pressure product) of the hearts subjected to ischemia was significantly lower compared to the oxygen perfusion control group (p = 0.0405). RPP in the group subjected to MF during ischemia was higher (19.4 ± 2.803) than in the group subjected to ischemia without MF (12.25 ± 4.694), the difference was statistically significant (p = 0.0123). In the group subjected to MF during ischemia the levels IL-6 and IL-10 were significantly lower than in the group subjected to ischemia without magnetic field (6.22 vs. 26.36 pg/ml and 9.48 vs. 19.61 pg/ml respectively).

The results of the experiment may suggest a beneficial effect of magnetic field on the ischemic heart and its potential cardioprotective properties. The differences observed in the levels of IL-6 and IL-10 may suggest that the magnetic field attenuates the inflammatory response provoked by ischemia.
Inhibition of matrix metalloproteinases by simvastatin protects hearts from ischemia-reperfusion injury

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Matrix metalloproteinase-2 (MMP-2) is rapidly activated in the heart within the first minutes of reperfusion following ischemia and contributes to myocardial stunning by cleavage of troponin I and MMP inhibitors protect hearts from ischemia-reperfusion injury [I/Ri; Circulation, 2002]. Several studies demonstrate the ability of statins to reduce the incidence of coronary heart disease and both acute and chronic mortality. They have several pleiotropic effects beyond lipid lowering, including anti-inflammatory actions. For example statins inhibit MMPs activity in macrophages and atherosclerotic plaques. Objective: The aim of this study was to determine the effects of simvastatin on functional recovery and MMPs activity in acute myocardial I/Ri.

Simvastatin (Merck; 0.2 µg/g body weight) or vehicle were given to rats twice (ip, 18 ± 2 and 3 ± 1 h before anaesthesia). Hearts were isolated and perfused at constant pressure either aerobically for 75 min or subjected to 20 min of aerobic perfusion, 20 min of global, no-flow ischemia followed by 30 min of reperfusion. The rate pressure product (RPP) was calculated and MMP-2 activity was measured in the coronary effluent.

Simvastatin prevented the impairment in mechanical function at 30 min of reperfusion seen in control I/Ri hearts, (Simvastatin + I/Ri:10.9 ± 1.2; I/Ri: 7.6 ± 2.1; Aerobic: 16.1 ± 2.5, n = 5, p < 0.05). MMP-2 activity after I/Ri in coronary effluent from simvastatin hearts was the same as that in aerobic hearts, while it was significantly higher in the I/Ri group (Simvastatin+I/Ri: 598.0 ± 178.8; I/Ri: 765 ± 118; Aerobic: 319 ± 140; n = 7, p < % 0.05).

Simvastatin inhibits MMP-2 activation in hearts subjected to I/Ri and improves their functional recovery. Further studies are needed to clarify the mechanism responsible for this effect.
Effect of antidepressants on antinociceptive action of opioids

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The World Health Organization recommends use of strong opioid based analgesics in long-term symptomatic treatment of intensive chronic pain. This recommendation is applied principally in tumor diseases and deeply progressed rheumatoid diseases. Prolonged serious illness may lead in patients to psychical disorders of various nature and extent, beginning with dysadaptive conditions through depressions and even to acute psychoses. Statistics show that depression symptoms are reported in 40% of patients treated for cancer. Therefore, an urgent need exists for parallel treatment of the basic ailment, combating pain and alleviating depression symptoms. Treatment of depression states involves application of medicines differing in chemical structure and mechanism of action, including three-ring antidepressants, such as amitriptyline, MAO inhibitors (such as moklobemid), selective serotonin reuptake inhibitors (SSRI), such as fluoxetine or norepinephrin reuptake inhibitor (reboxetine).

This paper provides an assessment of the effect of the above mentioned antidepressants on antinociceptive action of opioids.

The investigated opioids include the most commonly used analgesics, such as morphine, phentanil and buprenorphine. Research was conducted on male rats. Opioids were administered subcutaneously and antidepressants intragastrically. Pain threshold in investigated animals after analgesic drug administration was determined using an analgesimetric instrument before and after single and multiple premedication with antidepressants. Antidepressants were administered at different moments in time, depending on their pharmacokinetic properties.

Statistical analysis of the results allow to conclude that single premedication with antidepressants or their simultaneous use with opioids stimulates their analgesic action. In turn, long-term premedication with antidepressants leads to statistically significant decrease in the pain threshold value, pointing to increased pain experience of animals, associated with significant behavioral changes visible in the form of irritation, hypersensitivity or even aggressive symptoms. Opioid action was distinctly weaker.
Interaction of angiotensin-II receptor antagonists and conventional antiepileptic drugs in the maximal electroshock-induced seizures in mice

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Angiotensin-II (AII) receptor antagonists are most commonly used in the treatment of hypertension. They are also used as an alternative to angiotensin-converting enzyme (ACE) inhibitors in the management of heart failure or diabetic nephropathy.

The aim of the current study was to evaluate the effects of AII receptor antagonists (losartan and telmisartan), on the protective action of conventional antiepileptics (carbamazepine, phenytoin, valproate and phenobarbital) against maximal electroshock-induced seizures (MES) in mice.

Losartan (10, 20 and 50 mg/kg, ip) and telmisartan (5, 10 and 30 mg/kg, ip) did not influence the threshold for electroconvulsions. In the MES test, both drugs potentiated the protective activity of valproate. Losartan (50 mg/kg) decreased its ED50 value from 249.8 to 194.6 mg/kg while telmisartan (30 mg/kg) lowered the ED50 value from 249.8 to 190.6 mg/kg. Telmisartan (30 mg/kg) also enhanced the protective action of carbamazepine, decreasing its ED50 value from 10.5 to 7.8 mg/kg. Losartan did not influence the anticonvulsive efficacy of carbamazepine. Neither the action of phenytoin nor phenobarbital was affected by losartan or telmisartan.

In conclusion, Angiotensin-II receptor antagonists can potentiate the anticonvulsant activity of valproate and carbamazepine against MES-induced seizures. This effect may have be some significant for patients treated with these drugs.

The effect of antidepressants on delayed type hypersensitivity reaction

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A growing number of basic and clinical studies suggest a linkage between the brain and behavior and the immune system and resistance to disease. By suppression of immunological defense, behavioral factors, psychiatric disorders or psychotropic medications may contribute to increased susceptibility to a variety of diseases, including cancer.

In order to understand the interactions of antidepressant drugs with immunological function we evaluated the impact of antidepressant drugs on the immune system of the mouse. We report their effect on important host defense mechanism that plays a role in protection against tumor growth and virus infection – cell mediated immune response (CMI). Delayed type hypersensitivity reaction (DTHR) in the skin has been used to assess CMI in vivo. Using the contact sensitivity reaction on mice as a model system, we investigated the role of desipramine and fluoxetine in
the inhibition of DTHR. CBA mice were actively sensitized by application of picryl chloride (PC). Five days later, mice were challenged with PC. Antidepressant drugs, desipramine and fluoxetine, both at the dose of 10 mg/kg, were injected, ip daily for 14 days. The last drugs injection was 1 h before final ear measuring. Desipramine and fluoxetine inhibited ear swelling at 24 h by 53% and 49% respectively but it was impossible to transfer this immunosuppression to naive CBA recipients. Desipramine and fluoxetine are widely used in the treatment of the major depressive disorder and in other clinical settings.

Our observations indicate that such treatment may have potential deleterious effect on host defense.

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The effects of chronic corticosterone on rat behavior and hypothalamic-pituitary-adrenal axis activity

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The aim of this study was to analyze the influence of chronic corticosterone administration on rat emotional behavior and the HPA axis activity.

Rats were injected with corticosterone (daily injections of 5 or 20 mg/kg for 25 consecutive days) or vehicle (sesame oil). Behavioral effects in the contextual freezing test were compared with changes in plasma concentration of corticosterone and expression of CRF protein in different brain structures. Repeated administration of corticosterone (last injection 90 min before training session) enhanced freezing responses in the contextual fear test, and decreased plasma corticosterone concentration. Aversive context induced CRF production in the parvocellular and magnocellular neurons of the paraventricular hypothalamic nucleus (pPVN, and mPVN), central nucleus of the amygdala (CeA), cingulate cortex, area 1, 2 (Cg1, Cg2), primary and secondary motor cortex (M1, M2). In rats chronically pretreated with corticosterone this effect was attenuated in the pPVN, mPVN, enhanced in the CeA, and in the frontal cortex (Cg1, Cg2, M1, and M2).

The present results indicate the anxiogenic-like effects of chronic glucocorticoids administration. It is suggested, that chronic corticosterone treatment inhibits the hypothalamic-pituitary-adrenal axis. On the other hand, enhancement by chronic corticosterone of activity of CeA and the frontal cortex may lead to the improvement of memory of aversive events.
FK506 and cyclosporin A increase BDNF in ischemic astrocytes via Erk1/2 and calcineurin-dependent mechanism

Bożena Gabryel, Anna Pudełko, Jerzy Bernacki, Ewa Obuchowicz, Zbigniew S. Herman

The influence of two immunosuppressants FK506 and cyclosporin A on brain derived neurotrophic factor (BDNF) protein and its gene expression in astrocytes exposed in vitro to combined oxygen/glucose deprivation (OGD) was examined. We also investigated whether this effect is mediated through activation of extracellular signal regulated kinases 1 and 2 (Erk1/2) and/or attenuation of calcineurin activity. Additionally, the influence of both compounds on the protein expression of phosphorylated cAMP responsive element binding protein (CREB) transcription factor in nuclear extracts was determined.

In our study we used Western Blot technique to evaluate activity of ERK1/2 kinases, phospho-CREB and BDNF expression, radioassay to estimate calcineurin activity and RT-PCR method to measure BDNF mRNA expression.

On the 21st day cultures of astrocytes were subjected to OGD for 8 h in the presence of FK506 (10-1000 nM) or cyclosporin A (0.25–10 µM). It was shown that 1 mM FK506 or 0.25 µM cyclosporin A remarkably increased BDNF expression in astrocytes exposed to OGD. This protective effect of immunosuppressants was associated with calcineurin activity inhibition, increased expression of activated Erk1/2 kinases and phosphorylated CREB. Finally, we have also demonstrated that 1 mM FK506 (but not 0.25 µM cyclosporin A) significantly enhanced BDNF mRNA expression. FK506 and cyclosporin A ability to regulate BDNF via Erk1/2 and calcineurin dependent mechanism as well as their influence on CREB/CRE mediated transcription may contribute to their therapeutic efficacy in brain ischemia.

Locomotor activity of bupropion combined with conventional antiepileptic drugs in mice

Bartłomiej Barczyński, Katarzyna Mróz, Marian Wielosz, Piotr Tutka

Epilepsy and depressive disorders are very common in adult population. It has been proved that depression occurs more often in people with epilepsy. Key aspects of chronic treatment include careful balance between benefits and risks of treatment, since patients suffering from two or more coexisting diseases may be exposed to higher risk of adverse effects. Changes in the motor activity are common adverse effects observed during antiepileptic and antidepressant therapy. The aim of the study was to investigate the effect of bupropion (BUP), atypical antidepressant used as a first-line smoking cessation aid on the locomotor activity of mice treated with four conventional antiepileptics (carbamazepine – CBZ, valproate – VPA, phenobarbital – PB and phenytoin – DPH). The locomotor activity was tested using Digiscan Animal Activity Monitor System (Omnitech Electronics, Columbus, USA). All activity data were collected during three consecutive 15-min periods. Two representative motor indices were analyzed: total distance and ambulatory activity. BUP was administered for 14 days, every 12 h in a dose of 5 mg/kg, which did not change any locomotor index comparing to control (vehicle) group. The combined treatment of BUP and PB, DPH or VPA did not change both locomotor indices comparing to the control, BUP alone, and PB, DPH, VPA alone groups. Although the combined treatment of BUP and CBZ did not change ambulatory activity of
mice, it significantly reduced total distance traveled by animals in the first 15-min period, comparing to control, BUP alone and CBZ alone groups by 30, 23 and 26-fold, respectively. The combined administration of CBZ and BUP displayed serious dysfunction of exploratory activity of animals.

**Locomotor activity of bupropion combined with new antiepileptic drugs in mice**

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Depression is a common occurrence among epileptic patients. Bupropion (BUP), atypical antidepressant, strongly recommended for the treatment of tobacco dependence, has been shown to affect the anticonvulsive efficacy of antiepileptic drugs. In this study, we examined the effect of combined treatment of bupropion, and some new antiepileptic drugs (lamotrigine – LTG, topiramate – TPM and felbamate – FBM) on locomotor activity in mice. For this purpose, the electronically monitored locomotor activity test (Digiscan Animal Activity Monitor System, Omnitech Electronics, Columbus, USA) was used. Activity data were collected during two consecutive 15-min periods of time: the first one starting 15 min, and the second one starting 30 min after BUP administration. Two representative motor indices were analyzed: total distance and ambulatory activity. BUP was administered for 14 days, every 12 h in a dose of 5 mg/kg, which did not change locomotor indices comparing to control [vehicle] group. Combined treatment of BUP and LTG decreased total distance traveled by mice comparing to BUP alone group by 5-fold during first 15-min period and comparing to BUP alone and control group by 13 and 15-fold, respectively, during second period. The combined treatment of BUP and TPM showed reduction of total distance comparing to control group by 2-fold during second 15-min period. The combination of BUP and LTG also significantly reduced ambulatory activity during first or second 15-min periods comparing to control and BUP alone groups by 3 and 4-fold or 9 and 8-fold, respectively. The combined treatment of BUP and FBM did not change any locomotor indices during our study and was the only combination of drugs, which did not cause serious dysfunction of spontaneous locomotor activity of animals.

**The effect of gabapentin on memory functions in newborn rats exposed to tobacco smoke during fetal life**

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The source of neurocognitive impairments in epileptic patients are believed to have their background in both the etiology of the disease and applied pharmacotherapy. Antiepileptic drugs suppress unfavorable impact of seizures on patients’ functioning but on the other hand by reducing the responsiveness of neurons may have a negative effect on memory processes. Gabapentin (GBP) is a novel antiepileptic drug having normothymic properties. Its mechanism of action is associated with changes in amino acid concen-
trations and alterations within neurons, thus regulating the amount of excitatory and inhibitory neurotransmitters. Numerous references point to the increasing rate of maternal smoking during pregnancy has been shown to have a negative effect upon numerous cognitive functions, including memory processes, in both mothers and fetuses.

The aim of the study has been to investigate the effect of GBP on memory functions in newborn rats exposed to tobacco smoke during their fetal life. Pregnant animals were exposed to tobacco smoke (1500 mg/m³ of air) in a toxicological chamber for 3 weeks of their pregnancy. Female Wistar rats weighing 180–250 g were used in the study. GBP (25 mg/kg) was administered intraperitoneally 60 min before the test. The animals were subjected to Morris test for testing spatial memory.

GBP administered in a single dose and for a period of 7 and 14 days lowered values of escape latencies and lowered numbers of crossed quadrants compared to the control group. However on the day 21 GBP evoked no changes in spatial memory performance.

Treating epilepsy with novel drugs such as gabapentin allows both to control epileptic seizures and to improve impaired cognitive functions in the course of the disease. Improvement of originally impaired memory in animals (rats born by mothers exposed to tobacco smoke during pregnancy) indicates the possibility of modification of memory deficits accompanying epilepsy and other CNS diseases. This may further extend the spectrum of indications for gabapentin as a novel normothymic drug.

Effect of chronic treatment with perazine on lipopolysaccharide-induced interleukin-1β levels in the rat brain

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The aim of this study was to determine whether the chronic treatment with perazine alters lipopolysaccharide (LPS)-induced interleukin-1β (IL-1β) levels in the following rat brain regions: the hypothalamus, striatum, hippocampus and frontal cortex. Male Wistar rats were administered perazine dimaleate (15 or 30 mg/kg/day) in drinking water for 21 days. On day 22 LPS was injected, ip (125 µg/kg) 2 h before decapitation. Concentrations of perazine and its metabolites in plasma and brain were assessed by HPLC. The levels of IL-1β were determined using ELISA. Treatment with perazine (30 mg/kg/day) reduced LPS-stimulated IL-1β levels in the hypothalamus and a tendency to its decrease in the striatum and frontal cortex was observed.

This in vivo study suggests for the first time that long-term oral administration of perazine modulates reactivity of brain cells producing IL-1β.
Synthetic bastadins modify activity of ryanodine receptors in cultured cerebellar granule cells

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The aim of this work was to examine calcium transients in cultured cerebellar granule cells (CGC) induced by natural bastadin 10 and several synthetic bastadins. Although interactions of several natural bastadins, which are brominated macrocyclic bis-diaryl ethers derived from tyrosine, with the RyR1 isoform of the ryanodine receptor in sarcoplasmic reticulum has been described, their structure-dependent interference with the RyR2 isoform that is mainly expressed in the cardiac muscle and brain neurons has not been studied. The fluorescent calcium indicator fluo-3 and confocal microscope were used to evaluate changes in the intracellular Ca²⁺ concentration (Cai), and using pharmacological tools we assessed the involvement of ryanodine receptors. Our results demonstrated that apart from inactive BAST218F6 (a bisdesbromo analogue of bastadin 10), synthetic bastadin 5, and the synthetic bastadin analogues BAST217B, BAST240 and BAST268 at concentrations > 20 µM increased Cai, in a concentration-dependent, ryanodine- and FK506-sensitive way, with potency significantly exceeding the effect of 20 mM caffeine. Moreover the same active bastadins in the presence of ryanodine prevented at 5 µM concentration the thapsigargin-induced increase in Cai.

These results indicate that bastadins modify in a structure-dependent manner the activity of RyR2 in primary neuronal culture and provide new information about structure-related pharmacological properties of bastadins.

Topiramate effect on memory in alcohol preferring and nonpreferring rats

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There are few reports suggesting that topiramate (TP), a relatively new antiepileptic agent, can be of value in the alcohol dependence treatment [Johnson 2005, Krupitsky et al., 2007]. Since it is known that TP acts both as an enhancer on the GABA_A receptor and AMPA and kainate glutamate antagonist [Johnson 2005], and thus can cause cognitive impairment [Smith et al., 2006], therefore the aim of study was to assess the effect of TP on cognitive and behavioral activities in the animal model of alcoholism. The experiments were performed on ethanol 0.1 ± 0.8 g/kg/day) and 'non-preferring' (NP, 0.5 ± 'preferring' (PR, 4.7 g/kg/day) male Wistar rats treated subchronically (21×) with TP (50 mg/kg, ip) suspended in 1% Tween 80. After 21 days of TP administration short-term and long-term memories of rats were assessed using social recognition and passive avoidance tests, respectively. Moreover, the sedative activity, motor coordination, anxiety-related reaction were also established. TP produced the facilitation of short-term
memory in PRF rats, however, since vehicle-treated PRF rats showed impairment of social memory, the answer whether the effect was specific is not clear. The drug produced the slight significant anxiolytic activity in NPF rats, whereas such activity in PRF animals was not affected by TP administration. Moreover, the disruption of long-term memory and motor coordination after TP treatment in NPF animals were found. Concluding, due to the not clear activity of TP on memory in chronically ethanol-treated rats the TP beneficial effects in the treatment of alcoholism should be considered carefully.

The influence of long term administration of diferuloylmethane (CPE-014) on learning and spatial memory in the water maze in aged male rats

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Numerous studies have shown that diferuloylmethane is considered as a compound of a broad neuroprotective activity. It is able to diminish oxidative stress, inflammation and amyloid plaques formation in central nervous system. Data from preclinical and clinical studies suggest that it is also an antiplatelet, antiatherosclerotic and anticancer agent. The present study was undertaken to investigate the effect of long term administration of CPE-014 on the spatial navigation in naturally aged rats.

The influence of CPE-014 on learning and spatial memory was investigated in Water Maze Test. Wistar Albino Glaxo rats (600–800 g, 22-months old) received CPE-014 in standard diet in doses of 10 (C10, n = 8) and 50 (C50, n = 8) mg/kg/day for 2 months. Aged control animals (Con, n = 7) received standard diet only. The significant differences in mean latency to find the hidden platform over training were noted between experimental groups (C10: 28.27 ± 1.97s; p < 0.05, NK and C50: 25.6 ± 1.76s; p < 0.07; NK) and enhanced time spent in target quadrant (C10: 24.82 ± 3.42s; p < 0.05, NIR and C50: 22.88 ± 2.72s; p > 0.05, NIR) compared with Con (0.72 ± 0.36 and 16.57 ± 1.46s respectively). The latencies in repeated training on 8th day in C10 group (17.74 ± 3.0s; p < 0.05, NK) and C50 (17.5 ± 2.87s; p < 0.05, NK) were significantly higher than in control (33.78 ± 4.08s). In reversal platform test on 9th day the latency to find the hidden platform was the highest in control group (34.44 ± 4.46s) vs. C10 (31.99 ± 3.96s) and C50 (20.94 ± 3.27s). In the second memory test on 10th day no significant differences were noted between experimental groups in time spent in previous and second target quadrant but C50 group was more effective in crossings over previous target quadrant (2 ± 0.46; p < 0.05, NIR) vs. Con (0.57 ± 0.43).

The results indicate that chronic administration of CPE-014 potentiates the ability of learning and improves spatial memory in old rats.
The influence of long term administration of diferuloylmethane (CPE-014) on explorative and motor activity in the hole-board in aged male rats

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Behavioral studies have shown that diferuloylmethane, a broadly known neuroprotective agent, improves spatial navigation in aged animals. The present study was undertaken to investigate the effect of long term administration of CPE-014 on the explorative and motor activity in aged rats.

The influence of CPE-014 on behavior was investigated in Hole-Board Test. 22-months old male Wistar Albino Glaxo rats (600–800 g) received CPE-014 in standard diet in doses of 10 (C10, n = 8) and 50 (C50, n = 8) mg/kg/day for 2 months. Control groups: old (O-Con, n = 7) and young (Y-Con, n = 8) received standard diet only. Activity of animals was estimated as a number of insides, climbs and crosses between sectors of testing box area as well as time spent on moving and remaining in central quadrants in each trial.

Both experimental groups and young control showed significantly enhanced explorative activity measured as number of insides into holes and climbs. The mean number of insides over experiment was significantly higher in each experimental group (C10: 5.3 ± 0.64, p < 0.05, Newman-Keuls Test, NK; C50: 5.3 ± 0.6, p < 0.05, NIR) and in young control (Y-Con: 6.04 ± 0.73; p < 0.05, NK) compared with old control (O-Con: 3.14 ± 0.34). The mean number of climbs was also significantly higher in C10 (4.37 ± 0.44, p < 0.05, NIR), C50 (4.54 ± 1.0, p < 0.05, NIR) and in young control (4.38 ± 0.51; p < 0.05, NIR) vs. old control (O-Con: 2.14 ± 0.33).

Experimental groups of animals and young control spent more time on moving in the testing apparatus. The mean latency over experiment was higher in C10 (65.46 ± 9.21s), C50 (55.5 ± 6.49s) and Y-Con (66.13 ± 9.17s) vs. O-Con (49.95 ± 6.25s). No significant differences were noted between experimental groups compared with controls in number of crossings between nearly located sectors of testing box and in time spent in its central area.

The results indicate that chronic administration of CPE-014 improves explorative activity in old rats and may potentiate their motor activity.

Effect of D3 dopamine receptors blockade on the cognitive effects of angiotensin IV

Jan J. Braszko, Przemysław Wielgat, Anna Walesiuk

Our previous studies showed that D1 and D2 dopamine receptors are indispensable for the cognitive effects of angiotensin IV (Ang IV) and its des-Phe6 derivative des-Phe6-Ang IV to occur. As most neuroleptics currently used in the treatment of schizophrenia have variable D2/D3 dopaminolytic selectivity in this study we searched for the role of the D3 dopamine receptors in facilitating learning and improving memory actions of Ang IV and des-Phe6-Ang IV in rats.

For that purpose we evaluated recall of the passive avoidance behavior (PAB), object recognition (OR) memory, and the spatial working memory (WM) in...
rats treated with the intraperitoneal nafadotride (N[(n-butyl-2-pyrrolidinyl)methyl]-1-methoxy-4-cyano-naphthalene-2-carboxamide), a highly selective D3 receptor blocker and then by an intracerebroventricular Ang IV or des-Phe6-Ang IV. Separate groups of rats receiving the same treatments were run to check for the possible participation of unspecific motor (open field) or emotional (elevated “plus” maze) effects of our treatments in the results of the cognitive tests.

The results revealed Ang IV to express its improving recall of PAB, OR memory and WM action roughly similarly in all groups showing only minor or null significance of the D3 receptor blockade. Interestingly, in the nafadotride pretreated rats, des-Phe6-Ang IV beneficial effect on recall of the PA was weaker than that of Ang IV. Improvement of the spatial WM in an 8-arm radial maze, similar after Ang IV and des-Phe6-Ang IV, was not significantly affected by nafadotride. There were no motor and only minor anxiogenic effects of Ang IV and des-Phe6-Ang IV abolished by nafadotride in the former case.

In conclusion, this study points to the minor significance of the D3 dopamine receptors in the cognitive effects of Ang IV and interesting though unexplained inhibition by nafadotride of the des-Phe6-Ang IV effects.

Influence of zinc supplementation on effect of antidepressants in a chronic unpredictable stress (CUS) model in rats

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Zinc is an endogenous modulator of neuronal activity that might play an important role in the pathogenesis of depression. Recent studies have shown that zinc exhibits antidepressant-like activity in some models of depression in rodents. Our previous studies have shown that the footshock-induced fighting behavior was reduced in the rats subjected to chronic unpredictable stress (CUS). This test is used as the new experimental model of depression. Various antidepressant drugs given repeatedly prevented this kind of behavioral depression.

The aim of the present study was to evaluate the effect of prolonged treatment with zinc hydroaspartate and to examine if zinc supplementation could modulate the effect of antidepressants in CUS model of behavioral depression in rats. The experiments were carried out on male Wistar rats. Chronic stress (persisting of 16 days) was induced by the modified method described by Katz et al. Zinc hydroaspartate at the dose of 30 mg/kg/day or 15 mg/kg/day and antidepressant drugs (imipramine at the dose of 5 mg/kg/day, fluoxetine at the dose of 5 mg/kg/day, tianeptine at the dose of 6 mg/kg/day and moclobemide at the dose of 30 mg/kg/day) were administered once daily for 14 days. Antidepressant drugs were given (ip) 1 h before every stress session and zinc hydroaspartate (ip) 1 h before the antidepressants. The footshock-induced fighting behavior test was performed 48 h after the last session of the chronic stress.

It was demonstrated that in chronically stressed rats the number of fighting attacks was significantly reduced (by about 75%). Zinc hydroaspartate at the dose of 30 mg/kg/day, given alone, prevented the deficit in fighting behavior in chronically stressed rats. Neither antidepressant drugs nor zinc hydroaspartate at the dose of 15 mg/kg/day administered alone changed the intensity of fighting behavior in chronically stressed rats. However, when antidepressant drugs were given to the rats pretreated with zinc hydroaspartate (15 mg/kg/day) the deficit of fighting behavior was not observed.

The present results indicate that zinc similarly to antidepressants protects the rats against the CUS-induced behavioral depression. Moreover our findings suggest that zinc supplementation could potentiate the effect of antidepressant drugs.
Study on the role of central strychnine-sensitive glycine receptors in the pain perception in rats
Leszek Jędrusik, Izabela Jędrusik, Andrzej Plech

Glycine receptors mediate several functions of peripheral and central nervous system. The glycine/NMDA receptors modulate peripheral antinociceptive morphine effect in neuropathic pain in rats. The present study was undertaken in order to estimate the role of central strychnine-sensitive glycine receptors on pain perception. The experiments were performed on adult female Wistar rats which 7 days before experiments were implanted in chloralhydrate anaesthesia (300 mg/kg, ip) polyethylene cannulas into the lateral brain ventricle (icv). On the day of experiment unanaesthetized rats were icv injected with glycine at the range of doses of 10–200 nmol and antinociceptive effect was determined by a tail immersion test. It was found significant antinociceptive effect of glycine. Pretreatment rats with icv injection of equimolar dose of strychnine hydrochloride inhibited antinociceptive effect of glycine. Thus the role of strychnine-sensitive rats glycine receptors was confirmed.

Influence of bupropion and calcium channel antagonists on the memory-related response induced by nicotine in the elevated plus maze in mice
Marta Kruk, Barbara Budzynska, Grażyna Biała

There is evidence that cholinergic nicotinic system is involved in the modulation of memory. We investigated the possible implication of this system in memory-related behavior using the elevated plus maze test in mice. In this test, the time in which the mice took to moving from the open arm to the enclosed arm (i.e. transfer latency), was used as an index of memory.

Our results revealed that an acute administration of nicotine (0.1 and 0.5 mg/kg, sc) shortened the transfer latency in comparison with the saline-treated group. Moreover, we investigated the effects of bupropion and L-type voltage-dependent calcium channel antagonists on nicotine-induced memory-related behavior. Both, bupropion (10, 20 and 40 mg/kg, ip) and calcium channel blockers (nimodipine, flunarizine, verapamil, diltiazem – 5, 10 and 20 mg/kg, ip), injected 15 min prior to an acute injection of nicotine, dose-dependently reversed the improvement of memory induced by nicotine.

These findings indicate that cholinergic nicotinic system may play an important role in consolidation of memory and there is a close relationship between bupropion and nicotinic mechanisms. Bupropion is atypical antidepressant drug that may be an effective treatment for smoking cessation. In several behavioral tests, bupropion as a nicotine receptor antagonist blocked some of the effects of nicotine. Additionally, our results indicate that bupropion inhibited the improvement of memory induced by nicotine.

Our data show that neural calcium-dependent mechanisms may be involved in the modulation of memory-related response induced by nicotine, and that bupropion and calcium channel antagonists can promote the smoking cessation.
The influence of ACEA, a selective cannabinoid CB1 receptor agonist on whole blood serotonin concentration

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Through CB1 receptor cannabinoids modulate serotonin (5-HT) release in the CNS which is connected with some of their pharmacological effects, especially antidepressant activity. Serotonin plays many important biological functions also in periphery, particularly in the circulatory system, digestive system as well as in coagulation and endocrine glands secretion processes. It takes part in some diseases pathogenesis including hypertension, migraine, irritable bowel syndrome, asthma and hormonally active neoplasms. Cannabinoids possible influence on serotonin release in peripheral tissues may be clinically significant.

The aim of the present study was to investigate the influence of ACEA, a selective CB1 receptor agonist on whole blood (WB) and platelet-poor plasma (PPP) serotonin levels.

The experiments were carried out on male and female Wistar rats (250–300 g). ACEA (3 mg/kg, ip) was given alone and in combination with the selective CB1 receptor antagonist AM 251 (3 mg/kg, ip) 1 h before blood samples collection. Concentrations of serotonin in WB and PPP were measured by ELISA method.

ACEA significantly decreased concentration of serotonin in WB (to 61%, p < 0.02 vs control group) and its effect was blocked by AM 251. ACEA also reduced concentration of serotonin in PPP (to 62%) but the difference between control and ACEA groups was not significant.

Research results reveal that due to CB1 receptor stimulation, ACEA reduces serotonin contents in blood stream. This effect is probable the result of inhibition of serotonin release from tissue storage (enterochromaffin cells and mastocytes).

Virodhamine and abnormal cannabidiol relax the human pulmonary artery via the putative endothelial cannabinoid receptor

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Endocannabinoids (e.g. anandamide, virodhamine) modify vascular tone under physiological and pathophysiological conditions, including hypotension associated with hemorrhagic, endotoxic and cardiogenic shock. Their vasodilatory effects are partly mediated by a recently suggested so far not cloned endothelial cannabinoid receptor, which is distinct from classical CB1/CB2 receptors [for review, see Pacher et al., Pharmacol Rev, 2006]. However, it is so far unclear whether this vasodilatory cannabinoid receptor also occurs in human vessels. Thus, we investigated the effects of virdhamine on human pulmonary arteries. For comparison, the most potent synthetic agonist of the novel endothelial cannabinoid receptor, abnormal cannabidiol (abn-cbd), was used. The vasodilatory effects of cannabimimetics were examined on isolated human pulmonary arteries preconstricted with serotonin (5-HT; 1 µM) or with potassium chloride (KCl; 60 mM). Virodhamine and abn-cbd (both 0.1–100 µM) relaxed pulmonary arteries precon-
stricted with serotonin in a concentration-dependent manner (pD2 = 4.7, Emax about 95%; pD2 = 4.8, Emax about 110%; respectively). The vasodilatory effects of both cannabinoids were reduced by denudation of endothelium and they were less potent under KCl-induced tone. The relaxation evoked by virodhamine and abn-cbd in 5-HT-preconstricted, endothelium-intact vessels was diminished by the antagonist of the novel endothelial cannabinoid receptor O-1918 (10 µM) but not by the cyclooxygenase inhibitor indomethacin (10 µM).

In conclusion, virodhamine and abn-cbd relaxed isolated human pulmonary arteries by endothelium-dependent activation of the putative endothelial cannabinoid receptor. The relaxation is not related to prostacyclin but may involve K+ channels.

The effect of ebselen on neurons exposed to arachidonic acid and 4-hydroxynonenal in simulated ischemic conditions

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Release of free fatty acids, mainly arachidonic acid (AA), from membrane phospholipids is a predominant secondary mechanism producing neurotoxic effects of oxidative nature. As a polyunsaturated fatty acid, arachidonic acid can undergo peroxidation reactions with 4-hydroxynonenal (HNE) as one of the most important products.

We studied ebselen effects on AA and HNE toxicity in cultured rat cortical neurons in simulated in vitro ischemia by assessing viability and glutathione concentration. Rat cortical and cerebellar granule neurons, maintained in supplemented Neurobasal medium, were exposed to simulated ischemia (37°C, 3%O2, 5% CO2 with simultaneous glucose deprivation). Cultured cells were also exposed to AA or HNE. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and monochlorobimane were used for viability and glutathione concentration measurement, respectively.

Exposure of neurons to simulated ischemia caused a dramatic loss of cellular viability, exaggerated by co-exposure with AA or HNE. Ebselen attenuated their toxic effects. Both in normoxia and ischemia, ebselen increased the level of intracellular glutathione. 24-h exposure to AA or HNE elevated the glutathione level in normoxia and ischemia. In normoxia, pretreatment of AA- or HNE-exposed neurons with ebselen caused further significant elevation of glutathione level. In contrary, pretreatment of neurons with ebselen in ischemic conditions caused opposite effects. Ebselen normalized neuronal viability diminished by AA or HNE and increased glutathione level in normoxia and ischemia.

The results suggest that strengthening of intrinsic antioxidative mechanisms can protect against ischemia and/or arachidonic acid or 4-hydroxynonenal mediated injury to neurons.
Behavioral studies on 1-methyl-5-pyridin-3-yl-pyrroldidine-2-thione, a thioanalogue of cotinine

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Cotinine is a primary metabolite of nicotine that can exert measurable effects on certain types of behavior and cognition without exhibiting the toxicity usually attributed to nicotine [Terry et al., 2005]. The aim of this study has been to investigate some aspects of pharmacological profile of novel cotinine analogue, 1-methyl-5-pyridin-3-yl-pyrroldidine-2-thione (THCOT), since in our recent study THCOT was found to be a safe compound [Mikolajczak et al., 2005]. It is known that cotinine has been shown to exhibit biphasic effects in a variety of behavioral paradigms, therefore there was a need to expand dose-response studies.

Male Wistar rats were given a single injection of THCOT (0.1, 0.5, 1.0, 3.0, 30.0, 300.0 mg/kg, ip) and their sedative activity, motor coordination, anxiety-related reactions and cognitive function were assessed using actinometer, “chimney test”, elevated-plus maze and passive avoidance test, respectively. It was found that the improvement of motor coordination was shown in the doses of 0.1–3.0 mg/kg of THCOT, whereas in higher doses such effects were not observed. Anxiolytic THCOH activity was shown in the range of doses of 0.1–1.0 mg/kg, only. The compound affected neither (statistically significant) the cognitive function nor locomotor activity. However, after sub-chronic (21×) administration of THCOT (3.0 mg/kg, ip) the positive effects both on the motor coordination and long-term memory were found.

Concluding, it seems that effects of THCOT are shown to be biphasic similarly to cotinine, however applying the other tests for detailed assessment of THCOT-induced behavioral activity is thus an important subject for future investigation.

Activation of endocannabinoid system induces antidepressant-like effects in rats

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Activation of the endocannabinoid (EC) system results in euphoria and reduction of stress, anxiety and depressive symptoms in humans (Green et al., Drug Alcohol Rev., 2003). Moreover, a separate report indicates that enhancement of EC activity induces antidepressant-like effects in animals (Hill and Gorzalka, Eur Neuropsychopharmacol, 2005).

The present study examined the effects of the cannabinoïd CB₁ receptor agonist CP55,940 (0.03–0.3 mg/kg), the EC uptake inhibitor AM404 (0.1–3 mg/kg), the fatty acid amide hydrolase inhibitor URB597 (0.03–0.3 mg/kg) and the CB₁ receptor antagonist rimonabant (0.3–3 mg/kg) on immobility time in the forced swim test (FST) in rats. Moreover, the effects of CP55,940, AM404 and URB597 on the antidepressant-like activity of imipramine and citalopram in the FST were also examined.

We found that CP55,940 (0.1 mg/kg), AM404 (0.3–3 mg/kg) and URB597 (0.1–0.3 mg/kg) reduced the immobility time of rats. We also observed that the
anti-immobility effects of CP55,940 (0.1 mg/kg), AM404 (1 mg/kg) and URB597 (0.3 mg/kg) were blocked by rimonabant (3 mg/kg).

In another set of experiments we showed that the inactive dose of AM404 (0.1 mg/kg) potentiated antidepressant-like activity of the inactive doses of imipramine (15 mg/kg) or citalopram (30 mg/kg), while CP55,940 (0.03 mg/kg) and URB597 (0.03 mg/kg) enhanced the effect of imipramine only.

None of the drugs studied, given alone or in combination, increased the basal locomotor activity of rats.

Our results indicate that activation of the EC system induces antidepressant-like effects in the FST in rats, and that these effects are mediated by CB1 receptors.

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The Flash Visual Evoked Potential (FVEP) of rats after intracerebroventricular injections of β-endorphin

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The aim of this experiment was to find out the possible role of CNS opioid receptors in brain vision.

The experiments were carried out on Wistar rats a week after surgery performed for stereotaxically implants of the polyethylene cannula into lateral brain ventricle, an active electrode on the dura mater and the reference one, on the skull surface. Under chloral hydrate anesthesia, Flash Visual Evoked Potentials (FVEP) were recorded by an electrophysiologic apparatus interfaced with personal computer (LKC, USA), before and after saline or 1, 2, 5 μl injections into the lateral brain ventricular of 10 nmols of β-endorphin and 100 nmols of naloxone at the end of the experiment. Average of 150 responses every 5 min, during the 50 min observation for each dose of saline, beta-endorphin and naloxone were recorded. The shape of FVEP was completely changed after β-endorphine icv. The amplitude of the P2 wave was significantly decreased and after higher doses even reversed to negative values (~200%) for a long time. P2 latency was prolonged up to 145%. The amplitude of N1 wave decreased slightly, while the latency of N1 was significantly prolonged from 135% up to 145%. All negative effects of beta-endorphin on FVEP were rapidly eliminated by 100 nmols of naloxone injected icv. FVEP returned to normal state during 1 to 3 min. This is the first proof that beta-endorphin cause temporary disability of the central vision at the brain level. It is interesting which type of opioid receptor is mainly responsible for this effect.
Effect of chronically administrated tricyclic antidepressants on kynurenic acid – study in vivo

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Kynurenic acid (KYNA) is the only known endogenous broad-spectrum antagonist of excitatory amino acid receptors, displaying the highest affinity towards the glycine site of N-methyl-D-aspartate (NMDA) receptor. The cerebral synthesis of KYNA from its bioprecursor L-kynurenine is catalyzed by two distinct kynurenine aminotransferases (KAT I and KAT II). The disturbances of KYNA production have been linked to the occurrence of epilepsy, Huntington’s disease, Alzheimer’s disease, schizophrenia, AIDS-related dementia and others. Experimental data suggest that NMDA receptor antagonists display antidepressant-like activity in preclinical models. It was also demonstrated that chronic antidepressant therapy changes the function of NMDA receptor complex. The aim of this study was to evaluate the effect of chronic administration of tricyclic antidepressants: amitriptyline and imipramine on the brain formation of kynurenic acid in rats.

The animals were administered imipramine or amitriptyline (10 mg/kg, ip) for 1 or 14 days. Brain structures (cortex, hippocamp, striatum) were collected 24 h after the last injection of a drug and the level of KYNA and KAT’s activities were assessed. KYNA was quantified using HPLC system with fluorometric detector.

Single administration of imipramine and amitriptyline affected neither KYNA levels nor KATs activities in all studies brain structures. Chronic administration of imipramine and amitriptyline increased KYNA level in hippocampus, but not in cortex or striatum, up to 172% (p < 0.01) and 147.4% (p < 0.01) of control, respectively. Imipramine and amitriptyline enhanced also the KAT II activity in hippocamp up to 167.4% (p < 0.01) and 162.3% (p < 0.01) of control, respectively.

The data obtained here suggest that chronic antidepressant therapy might increase the hippocampal level of kynurenic acid and thus contribute to the modulation of neuronal plasticity and neurogenesis.

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Effect of repeated co-treatment with imipramine and metyrapone on the behavioral reactivity of central serotonin, dopamine and α1-adrenergic systems in rats

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It has been estimated that 30–40% of patients suffering from depression do not respond to a conventional therapy. Therefore, to improve the therapy, a combination of an antidepressant drug (AD) and a substance enhancing its effect, is used in the clinic. Since the hyperactivity of the hypothalamic-pituitary-adrenal axis may be a significant factor in the pathogenesis of depression, and since the lack of normalization of the axis activity throughout the therapy with ADs often correlates with the absence of their therapeutic effect,
it seems purposeful to study the effect of joint administration of ADs and glucocorticoid synthesis inhibitor in drug-resistant depression. It has been shown that metyrapone reveals antidepressant-like properties in the forced swimming test, and that co-treatment with ADs and metyrapone induces a more potent “antidepressant” effect than does treatment with either drug given separately.

The present study indicated that repeated co-treatment with imipramine (5 or 10 mg/kg) and metyrapone (50 mg/kg) (twice daily for 14 days; applied to male Wistar rats) either induced a more potent inhibition of the behavioral syndrome evoked by 5-HT_{1A}- and 5-HT_{2A} receptor agonists (8-OH-DPAT and (+)DOI, respectively) or did no change the action of amphetamine and quinpirole (dopamine D2/3 agonist) or phenylephrine (α1-adrenergic agonist) compared to treatment with either drug alone.

The above results support the hypothesis that co-treatment with ADs and metyrapone may evoke more effective antidepressant activity than does treatment with ADs alone, and that among other mechanisms, 5-HT_{1A}- and 5-HT_{2A} receptors may also play some role in this effect.

Impairement of recognition memory in interleukin (IL)-6 knock-out mice

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In addition to a key role of IL-6 in the function of immune system, IL-6 is released locally within the central nervous system (CNS) by astrocytes, microglia, and neurons. Several studies have been performed to estimate the role of IL-6 in the pathology and physiology of CNS, but still there is a little information about the role of endogenous IL-6 on cognition processes. To better understand this problem we evaluated the participation of endogenous IL-6 on cognition processes in mice lacking functional gene for IL-6. Examinations were carried out on transgenic male mice not expressing IL-6 [C57BL/6J IL-6–/–] (IL-6 KO) (13) and on wild type (WT) (14). The mice were housed in plastic cages, five animals per cage, in a temperature controlled room (22°C ± 1°C), under a 12 h light-dark cycle beginning at 7:00 h.

All testing took place during the first half of the light period (between 8.30–12.30).

To evaluate the influence of endogenous IL-6 on cognitive functions we used: object recognition test for recognition memory; “open field” test to estimate locomotor and exploratory activity and elevated plus maze test to determine anxiety-related behavior.

Deficiency of IL-6 significantly attenuated: recognition memory measured by the difference in exploration of the new object B and a duplicate of the familiar object A presented 1 hour later; locomotor and exploratory activity, and enhanced anxiety (in the elevated plus maze test knockout mice spent significantly longer time in closed arms as compared to WT controls).
Memory effect of clonidine in rats

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The aim of present study was to determine effect of peripheral (ip) administration of clonidine on rats memory in other experimental models and on their cognitive function. Experiments were performed on adult male Wistar rats, which were injected with clonidine hydrochloride at doses of 0.1 and 1 mg/kg. Rats memory was evaluated by test of active avoidance and by water maze test. For evaluation rats cognitive function exploratory and locomotor activity was determined by tests of open field and in the hole test. Rat irritability was assessed using a score of Nakamura and Thoenen.

The single clonidine injection neither impaired rats memory nor exploratory activity. Also rats irritability reflecting the degree of sedation was unchanged. On the other hand repeated administration of this drug delayed rats responses indicating the worsening memory and of cognitive function.

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Effect of haem arginate on convulsive effect of meso-tetra-4N-methyl-pirydyl-porphyrin in mice

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Among several symptoms of porphyrias are neurological manifestations such as seizures, paresis of the upper and lower extremities, paralysis and a wide spectrum of psychiatric disorders such as anxiety, depression, disorientation and delirium. The important drug used in treatment of acute porphyria is intravenous administration of haem arginate, which may correct haem deficiency and to restore the negative feedback control of haem. It was previously reported that synthetic porphyrine: meso-tetra-4N-methylpirydyl-porphyrin (P) induced neurotoxic effect manifested by clonic convulsions in mice and rats.

The present study was undertaken in order to estimate effect of haem arginate (Normosang – N, Orphan Europe, Warszawa, Poland) on P-induced convulsions in mice. Experiments were performed on male mice, Balby. Convulsive effect of P was determined using the following measures: percentage of mice with seizures, the number of seizure episodes/2 h, the latency time of the beginning P-induced seizure activity in mice and P-induced seizure activity of mice determined by score of Racine. N was applied at the range of doses 3–50 mg/kg, ip 20 min before P, which was next injected mol/kg, ip (59.3 mg/kg, ip).

It was found that N at the dose of 50 potentiated neurotoxic convulsive effect of P.

It is concluded that both determined porphyrins exert similar neurotoxic effect, which is expressed as pronounced convulsive effect.

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Role of NMDA receptor antagonism in the mechanisms of neuroprotection by 1,2,3,4-tetrahydroisoquinolines

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The aim of this study was to evaluate effects of endogenous tetrahydroisoquinoline derivatives, 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) and 1,2,3,4-tetrahydroisoquinoline (TIQ), on the activity of NMDA receptors in relation to their neuroprotective ability in vitro and in vivo. TIQ is known for its mild neurotoxic activity, whereas 1MeTIQ presents neuroprotective potential of the complex nature. Previously we noticed that 1MeTIQ inhibits NMDA receptors; here we tested the role of this phenomenon in the mechanism of its neuroprotection. Present experiments demonstrated that both 1MeTIQ and TIQ, inhibit the binding of [3H]MK-801 to rat brain membranes, the IC₅₀ value for TIQ being slightly higher. 1MeTIQ, and less potently TIQ, applied to primary cultures of rat cerebellar granule cells suppressed glutamate and NMDA-induced 45Ca uptake and glutamate-evoked increase in the intracellular Ca²⁺ concentration evaluated with fluorometric method. 1MeTIQ (0.5 mM) completely prevented the acute NMDA neurotoxicity, while 0.5 mM TIQ reduced it by 50%. In a model of perinatal hypoxia (brain hypoxia-ischemia in neonatal rats) 1MeTIQ significantly reduced brain damage in a dose of 50 mg/kg, injected, ip 3 times, every 2 h after the insult, whereas doses of 25 and 100 mg/kg were inactive or lethal, respectively. In the 1MeTIQ-treated animals we noticed no postischemic hypothermia or reduction of weight of the control hemispheres, which are typical effects of NMDA receptor antagonists in the immature brain. These results indicate that NMDA receptor antagonism in vitro is a common property of neuroprotective 1MeTIQ and neurotoxic TIQ, and point to complex mechanisms of their action.

Reinstatement of nicotine-induced conditioned place preference in mice

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In the present experiments, we used the conditioned place preference paradigm to study the extinction and reinstatement of extinguished nicotine place conditioning. Nicotine produced a place preference to the initially less-preferred compartment paired with its injections during conditioning (0.5 mg/kg, ip, three drug sessions). Once established, nicotine place preference was extinguished by repeated daily testing. Following this extinction phase, the reinstatement of place conditioning was investigated. For this purpose, rats were given one priming injection of either nicotine (0.5 mg/kg, ip) or morphine (10 mg/kg, ip). Our results show that both drugs are able to induce reacquisition of extinguished nicotine-conditioned place preference.

In the second step, in order to further examine the role of calcium-dependent mechanisms in drug relapse, we investigated the effect of two L-type calcium channel blockers, nimodipine (10 and 20 mg/kg, ip) and flunarizine (5 and 10 mg/kg, ip), on the ex-
pression of nicotine- or morphine-induced reinstatement of nicotine-induced place conditioning. Interestingly, we found that both antagonists, administered prior to the priming injections of nicotine or morphine, attenuated, in a dose-dependent manner, their reinstatement effect.

These findings support the hypothesis that similar neural calcium-dependent mechanisms are involved in nicotine- and morphine-induced reinstatement. Our data suggest that calcium ions and calcium channels play an important role in modulating the reacquisition of drug-seeking behavior following the extinction phase. This extinction/reinstatement model shows good predictive validity for the clinical efficacy of new compounds, including calcium channel antagonists, for the treatment of addiction, including nicotine dependence.

Brain level of FKBP-51, a glucocorticoid receptor cochaperone, is decreased in an animal model of depression

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A dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis activity is considered to be an essential factor involved in the pathogenesis of depression. Hypersecretion of glucocorticoids or disturbance in glucocorticoid receptor (GR) function observed in depression can be evoked by changes not only in GR density by also by alteration in GR chaperones or phosphorylation processes. Literature data indicated that among GR chaperons FKBP-51 (FK506-binding protein) is correlated with the frequency of depressive episodes and response to antidepressant drugs.

The aim of the present study was to investigate the effect of chronic treatment with antidepressant drugs on GR and FKBP-51 concentration in the hippocampus and prefrontal cortex in prenatally stressed rats (animal model of depression). In order to verify the model, immobility time in the forced swim test (Porsolt’s test) and the stress-induced blood corticosterone concentration were also determined.

It was found that prenatally stressed male Sprague-Dawley rats displayed high levels of immobility behavior in the forced swimming test and higher plasma corticosterone concentration one hour after acute stress. Treatment with imipramine, fluoxetine or mirtazapine for 21 days significantly attenuated these changes. Western blot study showed that the concentration of FKBP-51 in the prefrontal cortex of prenatally stressed rats was significantly lower than in control animals and chronic treatment with antidepressant drugs reversed this effect. Moreover, all three antidepressants under study attenuated also stress-induced changes in the GR concentration in the hippocampus.

The obtained data indicated that prenatal stresses changed GR signaling pathway by affecting the density of GR and FKBP-51 in discrete brain regions. Since FKBP-51 is known to inhibit GR function, too low concentration of this chaperone may be responsible for hyperfunction of GR in depression.

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Effect of noradrenergic neurons lesion as neonates on histamine level in the brain and reactivity of the central histamine receptors in adult rats

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DSP-4 [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine] is a neurotoxin which induces acute and selective degeneration of both central and peripheral noradrenergic nerve terminals in mammals. In rats, DSP-4 crosses the blood-brain barrier and produces long-lasting degeneration of the central noradrenergic neurons. Previously we showed that DSP-4 injected on the day 1st and 3rd of life of rats dramatically decreased noradrenaline (NA) level in the brain and modified function of the central serotoninergic, dopaminergic and GABAergic neurotransmitter systems in adult animals.

Newborn Wistar rats were injected with DSP-4 at 50.0 mg/kg, sc twice, on the day 1st and 3rd of postnatal life. Control newborn rats were injected with saline. When rats attained age of 8 weeks the level of NA was assayed in different parts of the brain (HPLC/ED). Beside the content of histamine (H) the brain was estimated by immunoenzymatic method. Then in a separate group of adult male rats with neonatally lesioned central noradrenergic system and control, several behavioral tests were performed. For this study three histamine receptor antagonists were used such as: S(+)-chlorpheniramine (for H1), cimetidine (for H2) and thioperamide (for H3). It was shown that neonatal lesion of the central noradrenergic decreased neurons significantly H level in the frontal cortex, and changed the central histamine receptors reactivity to antagonists (mainly H2 and H3) examined by behavioral methods in adult rats.

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Chronic stress changes expression and polysialylation of neural cell adhesion molecules in rat hippocampus

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Stress modifies molecular mechanisms involved in the modulation of brain structure and functions. Changes in synaptic interactions caused by stress lead to cognitive disturbances and exacerbate psychiatric disorders. Neural cell adhesion molecules (NCAM) belong to the family of the cell-surface glycoproteins that play a major role in cell-cell and cell-extracellular matrix interactions. Posttranslational modification of NCAM by addition of long α-2,8-polysialic acid chains changes their adhesive properties. NCAM and its polysialylated form PSA-NCAM are implicated in the modification and stabilization of synaptic contacts during memory formation.

In this study we investigated stress-related modulation of NCAM and PSA-NCAM in rat hippocampal synaptosomes. Wistar male rats were stressed for 21 days by the daily 2 h immobilization or injected with corticosterone (5 mg/kg) and trained for spatial mem-
ory in Barnes maze. Expression of NCAM and PSA-NCAM was assessed in hippocampal synaptosomes 24 h after the training by Western blotting.

Our results showed that repeated stress as well as corticosterone decreased statistically significantly ($p < 0.05$) amount of NCAM expressed in rat hippocampal synaptosomes. These changes appeared with learning impairment. In contrast, expression of PSA-NCAM were higher in stressed and corticosterone-treated rats than in control animals. Notably, corticosterone-induced alterations of NCAM and PSA-NCAM were greater than those induced by stress.

Our results indicate that changes in hippocampal NCAM and their polysialylation may play a role in the functional consequences of chronic stress.

Effect of AIDA in Morris water maze in diabetic rats

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Diabetes mellitus (DM) is associated with impairments of learning and memory. The central nervous system (CNS) complication of DM includes defects in hippocampal synaptic plasticity [Biessel et al., Diabetes, 1996; Kamal et al., Brain Res, 2006]. Abnormalities in glutamnergic neurotransmission have been identified in many CNS disorders.

The aim of this work was to investigate effect of antagonist [(RS)-1-aminoindan-1,5-dicarboxylic acid]- (AIDA) of group I metabotropic glutamate receptors (mGluRs) on acquisition and reference memory in the Morris water maze in intact and diabetic rats.

DM was induced by streptozotocin (STZ-60 mg/kg, iv) injection in rat. After 1 month of DM duration, the experiment was performed.

DM did not change the escape latency but decreased the time spent in the target quadrant in the transfer trial. AIDA (100 nmol/5 µl) did not influence acquisition of spatial learning and decreased locomotory activity in rats without DM. AIDA prolonged significantly the escape latency and distance in diabetic rats. AIDA did not affect the time spent in the target quadrant in the both studied groups.

In summary, AIDA impaired the spatial learning in water maze, but only in rats with DM.

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Memory effect of tamoxifen in rats

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Tamoxifen (TAM) a nonsteroidal antiestrogen is used for the treatment of breast cancer. Prolonged TAM application in woman suffering from breast cancer indicated that higher doses of this drug affect their cognition and memory Paganini-Hill and Clark, Breast Cancer Res Treat, 2000. TAM impairs memory consolidation and retrieval in mice Chen et al., Pharmacol Biochem Behav, 2002. The present study was undertaken in order to determine effect TAM on memory function in rats. Experiments were performed on adult, intact, Wistar rats of both sexes. TAM was injected intraperitoneally (ip) at doses of 1, 10 and
50 mg/kg. Memory consolidation was determined in the test of active avoidance and in a water maze test. Moreover rats exploratory and motor activity was determined in an open field and a hole test.

It was found that single TAM injections were without any significant effect on rats memory and their locomotor and exploratory activity. The repeated TAM injections at the higher dose of 50 mg/kg, ip impaired memory consolidation in the test of active avoidance in male as well as in female rats. Intramuscular injection of Estradiol depot (10 mg/kg) together with TAM prevented TAM effect.

Conclusion: TAM-induced impairment of memory was confirmed but only after it repeated dosing of the higher dose of TAM. This effect depends on the estrogen deficiency.

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Influence of vigabatrin on dexamethasone-induced neurotoxicity: behavioral and morphological studies

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Recent years of studies have evidently showed that long-term treatment with glucocorticoids (GCs), prolonged stress or ischemia induced neuronal damage. Furthermore, elevated levels of endogenous corticosteroids are known to be toxic to the CA1 and CA3 subfields of the hippocampus and in the striatum and reversibly decrease specific elements of memory performance. Moreover, GCs potentiate stress- or ischemia-induced accumulation of excitatory amino acids (EAA) in the extracellular space of hippocampus and by facilitating the glutamate/Ca2+ cascade endanger hippocampal neurons damage.

A similar alternation was also noted following the administration of dexamethasone (DEX- a synthetic GCs receptor agonist), which induces mood disorders including psychosis in some patients.

Some authors suggested the neuroprotective effect of the antiepileptic drugs such as phenytoin or the new generation of these drugs, e.g. vigabatrin, tiagabine or topiramate because of their γ-aminobutyric acid (GABA) neurotransmission or by potentiation of reduction of brain excitatory amino acids levels.

The purpose of the present study was to investigate the effect of vigabatrin on neurotoxic effect of DEX.

The experiments were carried out on male Albino Swiss mice (25–30 g) for 20 days. Vigabatrin (75 mg/kg/day, ip) was administered 4 h before DEX (80 mg/kg/day, ip) each day. The long-term memory acquisition (the step-through passive avoidance test), the motor performance (“chimney” test) and morphological study were evaluated 10 days after the drugs administration. Moreover, the body weight and the lethality of mice were controlled each day.

We have also examined the morphology of neurons in the dorsal hippocampus in slides stained with cresyl violet. Quantitative analysis of morphological changes was carried out by counting the number of damaged neurons using a projection microscope.

The results of our study have shown the prolongation of climbing time in “chimney” test, decrease of the retention time in the memory task, the reduction of the body weight and the increase of the lethality of mice treated chronically with DEX. DEX at the dose used evoked histological damage of hippocampal neurons, especially in the CA3 field. The neuropathological changes were characterized by some shrinkage and condensation of nuclear chromatin. Vigabatrin, administered alone, changed neither the be-
behavior of mice nor the body weight nor the lethality in comparison with control group. In mice treated with DEX, vigabatrin reduced the climbing time in “chimney” test, improved acquisition of memory and did not influence the lethality or body weight in comparison with DEX alone. DEX-induced damage of hippocampal pyramidal neurons was reduced in animals treated with vigabatrin.

The above findings suggest that vigabatrin could prevent the neurotoxic effects induced by DEX.

Session: PEPTIDES AND HORMONES

Is neuropeptide FF involved in rewarding action of cocaine?

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Neuropeptide FF (FLFQPQRF-NH₂, NPFF) is called opioid-modulating peptide because given intracerebroventricularly (icv) inhibits antinociception induced by morphine, but given intrathecally potentiates this morphine effect. The pharmacological actions of NPFF are mediated by two G-protein-coupled receptors present in various brain structures. Our previous studies suggested that NPFF suppressed morphine-induced sensitization to its locomotor effects and morphine-induced place preference.

The aim of the present study was to indicate whether NPFF is also involved in rewarding effects of cocaine. We studied an influence of NPFF on the expression of sensitization to locomotor effect of cocaine in mice, and the expression of cocaine-induced place preference (CPP) in rats. Sensitization to cocaine was induced by five intermittent administrations of cocaine (10 mg × kg⁻¹, sc) (Kuribara 1995). On the test day, NPFF (5, 10, 20 g, icv) was given just before the challenge dose of cocaine (10 mg × kg⁻¹, sc), and locomotor activity was measured for 2 h. Cocaine-induced conditioned place preference (CPP) was induced according to the method of Bardo et al. [1995]. The conditioned place preference schedule consisted of a pre-conditioning phase (1 day), conditioning phase (4 days) and testing phase (1 day) expression of cocaine µ. The effect of NPFF (5, 10, 20 g, icv) on the expression of cocaine CPP was measured on the test day.

Our results indicated that NPFF decreased the expression of cocaine-induced sensitization to its locomotor effect and cocaine-induced CPP. These studies suggest that NPFF is involved not only in the morphine-, but also in the cocaine-induced rewarding effects.
Antihypertensive effect of mistletoe extracts and their fractions and natriuretic peptide level and plasma renin activity in rats

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Mistletoe exerts hypotensive, cardiotonic, vasodilative and antineoplastic effect. Hypotensive effect of *Viscum album* preparations has been described by many authors, yet the reports on their efficacy in hypertensive patients are equivocal.

In the presented study we have shown that Intractum *Viscum album* (IVA) reduced blood pressure in normotensive rats (WKY), as well as in rats with renal hypertension (RHR) and with genetically determined hypertension (SHR). Of many fractions obtained through solvent extractions the most potent hypotensive effect could be observed for aqueous fraction. When further extracted, the latter yielded 3M subfraction which contained such phenolic compounds as malic acid and caffeoylquinic acid with its isomers. This subfraction was administered orally to RHR rats in the dose of 200 mg/kg for 14 days. The analysed subfraction significantly reduced the level of natriuretic peptide (pro-ANP) and plasma renin activity in the analysed group of animals.

It seems plausible that tinctures made of fresh mistletoe leaves exert mild hypotensive effect in various types of hypertension and the hypotensive effect is probably induced by phenolic compounds found in the plant.

Prolonged antiuterotonic effect of naphthylalanine analogues of arginine vasopressin modified in position 1 with 1-mercaptocyclohexane acetic acid on the isolated rat uterus

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We found previously that peptides of naphthylalanine in position 2 of arginine vasopressin (AVP) have a potent rat antiuterotonic activity *in vitro*.

The purpose of this study was to examine the two analogues of AVP substituted in position 1 with 1-mercaptocyclohexane acetic acid (Cpa) on uterus contraction of rats *in vitro*.

Antiuterotonic activity was assayed on the isolated rat uterus by the procedure of Holton. Van Dyke-Hastings solution supplemented with Mg²⁺ with the modification described by Munsick was used as the bathing fluid. Wistar rats were premedicated by oestradiol benzoicum (0.8 mg/kg, *im*) 24 h before experiment. Laparotomy was carried out under inactin anesthesia (125 mg/kg, *ip*). The uterine contraction was determined using isometric transducer and quantified by digitizing the area under the response curve. Antagonistic activity was measured by Schild’s method and expressed as pA₂ value.

It was shown, that high antiuterotonic activity as compared to AVP effects in uterus smooth muscle contraction test was evoked by [Cpa1,D-1-Nal2]AVP. The value of pA₂ was 8.6. The antiuterotonic activity of [Cpa1,L-1-Nal2]AVP was moderate (pA₂ 7.26). The half time of 50% sensitivity of uterus contraction was 2.8 h and 1.32 h for [Cpa1,D-1-Nal2]AVP and [Cpa1,L-1-Nal2]AVP.
Ebselen attenuates oxidative stress in ischemic astrocytes depleted of glutathione. Comparison with glutathione precursors

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Ebselen is a seleno-organic compound which mimics the activity of the endogenous glutathione peroxidase (GPx) and phospholipids hydroperoxide GPx. The drug acts also as an oxidant at redox modulatory sites within several ligand-gated ion channels e.g. the nicotinic acetylcholine receptor and the NMDA subtype of glutamate receptor.

In the present study the effect of ebselen towards astrocytes degeneration caused by exposure to simulated in vitro ischemic conditions and simultaneous depletion of glutathione (GSH) was investigated. Depletion of GSH was induced by 24 h pretreatment with L-buthionine-(S,R)-sulfoximine (BSO). In this experimental paradigm the effects of ebselen (1–40 µM) on apoptosis, mitochondrial function, reactive oxygen species (ROS) production, intracellular GSH level and mitochondrial transmembrane potential (MTP) were examined. In addition, the antioxidant potential of ebselen with cystine and methionine as a precursors of GSH synthesis as well as with GSH ethyl ester was also compared. The study demonstrated that toxicity of simulated ischemia conditions were enhanced when intracellular GSH was depleted. Cytotoxicity was prevented by treatment with ebselen, especially at concentrations of 20 and 40 microM. The study has shown that antiapoptotic effect of ebselen is associated with strong antioxidative properties, preservation of MTP and possibly conservation of mitochondrial GSH during cytoplasmatic GSH depletion caused by oxidative damage. Also, promoting GSH synthesis by the delivery of substrates as cystine or inhibition the efflux of GSH by methionine may be a powerful strategies to minimizing cell damage to the nervous tissue after ischemia.

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Study on the role of nitric oxide in the mechanism of
antinociceptive effect of heptapeptide [2-8]-leucopyrokinin
([2-8]-LPK) in rats
Monika Rykaczewska-Czerwińska¹, Danuta Konopińska², Andrzej Plech¹

Heptapeptide [2-8]-leucopyrokinin ([2-8]-LPK) is a synthetic analog of octapeptide leucopyrokinin
(LPK) (pGlu-Thr-Ser-Phe-Thr-Pro-Arg-Leu amide) a neuropeptide of Madeira cockroach (Leucophaea
maderae) and of other several insects. It was previously reported that both peptides: LPK and [2-8]-LPK
injected into the lateral brain ventricle exert evident antinociceptive effect in rats. Antinociceptive effect
of either native LPK or its analogue [2-8]-LPK was mediated by central opioid receptors as was blocked
by naloxone an unselective opioid antagonist and as well as µ- and δ-opioid receptors blockers.

The present study was undertaken in order to determine a role of NO in mechanism of [2-8]-LPK-
induced analgesia. The experiments were performed on adult male Wistar rats, which were implanted with
polyethylene cannula into the right lateral brain ventricle (icv) using the same method as in our previous
study. Antinociceptive effect of two doses LPK of 5 and 10 nmols icv was mediated by central opioid receptors as was blocked by naloxone an unselective opioid antagonist and as well as by µ- and δ-opioid receptors blockers.

The present study was undertaken in order to determine a role of NO in mechanism of [2-8]-LPK-
induced analgesia. The experiments were performed on adult male Wistar rats, which were implanted with
polyethylene cannula into the right lateral brain ventricle (icv) using the same method as in our previous
study. Antinociceptive effect of two doses LPK of 5 and 10 nmols icv was mediated by central opioid receptors as was blocked by naloxone an unselective opioid antagonist and as well as by µ- and δ-opioid receptors blockers.

The results of present study indicate the role of NO in the mechanism of [2-8]-LPK-induced antino-
ciceptive effect in rats, which is produced by neuronal NOS.

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Nitric oxide modulates the amphetamine effect on (3H)glucose uptake in the brain of rats prenatally exposed to lead
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Lead (Pb) is a highly neurotoxic agent in both mammals and human. Many studies have demonstrated
that exposure to environmental lead adversely affects a variety of the central neurotransmitter systems in-
cluding dopaminergic one.

Glucose is a main source of energy for the brain. In this study the effect of amphetamine, a psychostimu-
ant and neurotoxin for the dopaminergic system on (3H)glucose uptake in the brain of adult rats prena-
tally exposed to lead, and the role of nitric oxide...
(NO) in it was examined. Wistar pregnant rats were $3H_2O \times Pb(CH_3COO)_2$ consumed in their drinking water 250 ppm of lead throughout their entire pregnancies. On the day of parturition the lead containing water was replaced by tap water, and the offspring remained with their mothers for 21 days. Control pregnant rats consumed water without metal. Adult male offsprings from both groups (lead exposed and control) were pretreated with 7-nitroindazole (nNOS blocking agent) 10.0 mg/kg, ip or saline 1.0 ml/kg, ip and 30 min later with amphetamine 1.0 mg/kg, ip. Then after 30 min all rats were injected Ci/kg, ip. Fifteen minutes later with 6-3H-D-glucose (Amersham) 500 animals were sacrificed, samples of the brain were excised and radioactivity was measured in liquid scintillation counter, and expressed in DPM (Desintegration Per Minute) per 100 mg of wet tissue.

The results have shown that NO modulate (3H)glucose uptake in the brain after amphetamine injection mainly in the rat prenatally exposed to lead.

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**Session:** MOLECULAR PHARMACOLOGY

Influence of histamine, thioperamide and imetit on the proliferation of B16 mice melanoma cell line *in vitro*

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The aim of the study was to evaluate effect of histamine, thioperamide and imetit upon the proliferation of B16F0 mice melanoma cells *in vitro*. Thioperamide and imetit were used in concentrations of: 100 µM, 10 µM, 1 µM, and 0.1 µM, histamine additionally in the 0.01 µM. Experiment involved: establishing the culture; preparing suspension of $10 \times 10^4$ cells/mL (series 1) or $2 \times 10^4$ cells/mL (series 2); counting cells after 24 h – preliminary control (Cp); addition of the substances; counting cells after 72 h – final control (Cf) or final result (Cx) if the studied substances were added. Test value (TV) and test index (TI) were calculated: $TV[\%] = (Cx-Cp)/(Cf-Cp) \times 100$; $TI[\%] = TV \times Cp/Cx$. If TI was lower than −80% cells were sensitive, if ranging between −40% and −80% cells were slightly sensitive, while with values exceeding −40% cells were not sensitive to studied substance. According to these criteria B16F0 mice melanoma was not sensitive to the studied compounds, independently from the concentration or culture density. However, the average increase in cell number in the cultures with thioperamide (100 µM) was 27.3% (series 1) and 35.7% (series 2) of that in respective control cultures. In the culture with imetit (100 µM) was 9.1% (series 1) of control. No significant influence of histamine on cell proliferation was observed in series 1. Slight acceleration of B16F0 melanoma cell proliferation (185.7% of that in control) was observed in series 2 (histamine 1 µM). Further exact studies on cellular mechanisms of such action are necessary.
Novel human serotonin3 receptor subunits 5-HT3C, 5-HT3D and 5-HT3E: pharmacological and functional characterization

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The 5-HT3 receptor, a pentameric channel complex permeable to Na+, K+ and Ca2+, is the only ligand-gated ion channel within the family of serotonin receptors. 5-HT3A and 5-HT3B subunits, appropriately assembled to form functional channel complexes, are well characterized. Binding of an agonist to the 5-HT3 receptor leads to a fast excitatory response. Some of us recently reported on the cloning and expression analysis of the human 5-HT3 receptor like genes HTR3C, HTR3D and HTR3E [Niesler et al., Gene, 2003].

The present study in HEK 293 cells aimed at examining by means of immunofluorescence and immunoprecipitation of recombinantly expressed proteins whether the subunits derived from the genes and the subunit isoform 5-HT3Ea are able to form 5-HT3 receptors. Furthermore, ligand binding studies as well as calcium influx analyses were performed to explore whether they modulate 5-HT3 receptor function. We found that each of the candidates co-assembles with 5-HT3A. The 5-HT3C, D, E and Ea subunit alone cannot form functional receptors. Co-expression with 5-HT3A, however, results in the formation of functional heteromeric complexes with different 5-HT efficacies. Potencies of two agonists and antagonists at homomeric 5-HT3A and heteromeric complexes were nearly identical. However, the efficacy of 5-HT with respect to 5-HT3A/D and 5-HT3A/E receptor-mediated responses was increased. This is consistent with the increased surface expression compared to 5-HT3A receptors. In contrast, 5-HT3A/C and 5-HT3A/Ea receptors exhibited decreased 5-HT efficacy.

In conclusion, the novel 5-HT3 subunits can form heteromeric 5-HT3 receptors whose functional properties are quantitatively different from homomeric 5-HT3A receptors.

Photocytotoxic effect of diamino acid protoporphyrin IX derivatives on human cancer cells in vitro

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Protoporphyrin photosensitizers are used more and more often in diagnostics and tumor therapy. The photosensitizers, diamino acid protoporphyrin IX derivatives are also used in the photodynamic therapy.

In the present study, two diamino acid protoporphyrins derivatives: diarginine protoporphyrin IX (PPArg2) and diarginine di-(N-alanyl)-protoporphyrin IX (PP(Ala)2Arg2) were investigated. Photocytotoxic properties of PPArg2 and PP(Ala)2Arg2 were examined in vitro on two types of human tumor cells culture: KB cells of oral cavity cancer and EJ-138 cells of urinary bladder cancer.

The cells culture were grown in complex media RPMI 1640, containing 10% of fetal bovine serum, at 37°C in a humidified atmosphere of 5% CO2 in air. Concentration of protoporphyrin IX derivatives added into cell culture was 1 × 10^{-5} and 1 × 10^{-4} mol/l. Estimation of the protoporphyrin content inside cells was
determined by fluorescent method (wavelength 515 nm). To destroy the cancer cells, the cultures were exposed to red diode laser at lambda = 632 nm, in 30, 60 and 90 min (6.5; 13 and 19.5 J/cm².

Mortality of the cancer cells was estimated by spectrophotometric determination.

Concluding, PPArg2 and Pp(Ala)2Arg2 penetrate into human tumor cells KB and EJ-138 cultured in vitro. Results indicated that photocytotoxic effect was much more greater for PPArg2 than Pp(Ala)2Arg2 and raised proportionally to laser absorbed energy by cancer cells culture in vitro.

Modulation of MRP1 transport activity by genistein and it’s synthetically modified derivatives

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Genistein (5,7,4’-trihydroxyisoflavone) is isoflavone abundant in soybean and found in other plants of Fabaceae. It plays an important role as a phytoestrogen, and shows a wide variety of other pharmacological effects in human cells. The most important are: inhibition of tyrosine kinase and chemoprotectant activity against cancer and cardiovascular disease. Genistein recently became a subject of many studies when those new biological activities (as chemoprevention against cancer and cardiovascular disease) were found.

Basing on the beneficial biological effects of genistein some efforts are made to synthesize the derivatives of this isoflavone, which would be even more potent than genistein itself. In present work we studied the influence of genistein and it’s synthetic derivatives (7-O-(4-methoxybenzyl)-genistein, 7-O-(4-cyanobenzyl)-genistein, 7-O-(3-chlorobenzyl)-genistein) on transport activity of multidrug resistance-associated protein (MRP1). This activity was investigated in human erythrocytes used as a cell model expressing MRP1 in plasma membrane. The fluorescent probe, BCECF 2’,7’-bis-(3-carboxy-ethyl)-5-(and-6)-carboxyflurorescin, was applied as a substrate for MRP1 multidrug resistance transporter.

The studied isoflavones showed the inhibitory influence on transport carried out by MRP1. 7-O-(4-methoxybenzyl)-genistein and 7-O-(4-cyanobenzyl)-genistein (but in higher molecular concentration – about 100 µM) showed higer inhibitory potency than natural isoflavone (genistein). The inhibitory activity of 7-O-(3-chloro benzyl)-genistein was however similar to the activity of genistein. Since MRP1 inhibition could be caused either by direct interaction of isoflavonoids with protein or by indirect perturbation of membrane properties some further investigations are necessary to elucidate the molecular mechanism underlying the inhibitory activity of genistein and its derivatives.
Antiapoptotic action of excitatory neurosteroids in human SH-SY5Y cell line

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Excitatory neurosteroids, dehydroepiandrosterone (DHEA) and pregnenolone (PGL), are precursors of steroid hormones which influence the excitability of neurons. These neurosteroids occur at high concentration in the brain both in the free form and as sulfate esters. DHEA(S) and PGL(S) are important regulators of the central nervous system function and may be involved in processes of neuronal cell survival. The aim of the present study was to estimate the neurosteroid effects on necrosis and apoptosis induced by hydrogen peroxide and staurosporine in SH-SY5Y human neuroblastoma cells. It has been found that DHEAS (0.1–10 µmol) inhibited the hydrogen peroxide toxicity in a concentration-dependent manner whereas DHEAS (10 and 100 µmol) was active only at higher doses. PGL and PGLS showed neuroprotective effects only at the lowest concentration. Next part of this study showed that DHEAS, DHEA and PGL significantly antagonized effects of staurosporine on both caspase-3 activity and the mitochondrial membrane potential, whereas PGLS inhibited the staurosporine-induced changes in both apoptotic parameters only at the lowest concentration. Antiapoptotic properties of neurosteroids were positively verified by Hoechst staining. Furthermore, as shown by calcein assay, DHEA, DHEAS and PGL increased viability of staurosporine-treated cells and these effects were attenuated by specific inhibitors of phosphatidylinositol 3-kinase (wortmannin) and extracellular signal-regulated protein kinase (ERK) – mitogen activated protein kinase (PD 98059). These data indicate that neurosteroids prevent SH-SY5Y cell damage related to oxidative processes and activation of mitochondrial apoptotic pathway. Moreover, neuroprotective effects of DHEA, DHEAS seem to depend on PI3-K and ERK/MAPK signaling pathways. It can be suggested that at physiological concentrations all studied neurosteroids participate in the inhibition of neuronal apoptosis.

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Cannabinoids attenuate the activity of thyroid parafollicular cells in rats

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Previous reports have shown that delta-9-tetrahydrocannabinol, the major psychoactive cannabinoid component of marijuana, is able to inhibit the release of T3 and T4. The aim of this study was the evaluation of the influence of a single, ip injection of a stable analogue of anandamide – R-(+)-methanandamide (2.5 mg/kg) or CP 55,940 (0.25 mg/kg), an exogenous agonist of
CB1 receptors, on the activity of parafollicular cells in male Wistar rats. Four hours after a single injection of each cannabinoid or vehicle, the animals were anaesthetized and blood was taken from the abdominal aorta to determine calcitonin plasma concentration by RIA. Subsequently, the animals were thyreoidectomized. Ultrastructural and immunohistochemical study of thyroids, using specific antibodies against calcitonin and CGRP were performed. The intensity of immunohistochemical reaction for calcitonin and CGRP in thyroid of rats injected with cannabinoids was stronger in majority of C cells as compared to the controls. Ultrastructural pattern of parafollicular cells after a single injection of R-(+)-methanandamide or CP 55,940 showed irregular shape of the nuclei and dilated ergastoplasmic sacs with a small number of ribosomes in both examined groups. The quantity and electron density of secretory granules within C cells increased in comparison to the controls. These microscopic changes were accompanied by a significant decrease of calcitonin plasma concentration in rats treated with both cannabinoids.

These results indicate that a single injection of R-(+)-methanandamide or CP 55,940 attenuates the thyroid parafollicular cells activity.

The effect of μ- and δ-opioid receptors stimulation by specific agonists given icv on FVEP in rats

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Recently, we have found that β-endorphin, a potent opioid peptide enormously decreased and completely changed the shape of Flash Visual Evoked Potential (FVEP) of rats. The aim of this study was to find which subtype of opioid receptors is responsible for this effect.

The experiments were carried out on 30 Wistar rats stereotaxically implanted with polyethylene cannula into lateral brain ventricle (icv) and with the electrodes active on the dura mater and reference one, on the skull. A week later the FVER were recorded by LKC electrophysiologically interfaced personal computer system (USA), with Ganzfield stimulation of both mydriatic eyes, under chloral hydrate anesthesia.

The μ-receptor agonist DAMGO ([D-Ala², N-Me-Phe⁴, Gly-ol⁵]-Enkephalin) and Δ agonist DPDPE ([D-Pen², ⁵]-Enkephalin) were injected icv in 10 µl saline volume. DAMGO in a doses of 1, 5 and 10 nmol changed the shape of FVEP proportionally to the dose used, similarly to the effect of β-endorphin, which was blocked by equivalent doses of naloxone. DPDPE in doses of 1, 5, 10, 20 and 100 nmol had no influence on FVEP. Thus, stimulation of opioid μ receptors causes depression and total destruction of FVEP, particularly it’s peak P2 and probably deep deprivation or even temporary loss of vision in rats, likelihood in human too.
Methanandamide-induced, cannabinoid receptor-independent inhibition of α7-nicotinic acetylcholine receptor-mediated response in vivo

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Methanandamide (MAEA), the stable analogue of the endogenous cannabinoid (CB) receptor agonist anandamide, has been shown to inhibit the function of the α7-nicotinic acetylcholine receptors (α7-nAChRs) in Xenopus oocytes in a CB receptor-independent manner [Oz et al., J Pharmacol Exp Ther, 2004]. Here we examined whether this inhibition is detectable in vivo. We determined the tachycardiac response to electrical stimulation of preganglionic sympathetic nerves by the pithing rod or to iv nicotine (0.7 µmol/kg) activating nicotinic acetylcholine receptors (nAChRs) on the cardiac postganglionic sympathetic neurons. The rats were anaesthetized, pithed, vagotomized and treated with atropine. When anaesthetized with pentobarbital, but not in urethane-anaesthetized rats, 3 µmol/kg MAEA inhibited the electrically-induced tachycardia by about 15% (blocked by the CB1 antagonist AM 251). Urethane-anaesthetized rats, thus, are suitable to study the MAEA-induced CB1 receptor-independent inhibition of nicotine-evoked tachycardia. The α7-nAChR antagonist methyllycaconitine (MLA; 1 µmol/kg) and the nonselective nAChR antagonist hexamethonium (30 µmol/kg) decreased the nicotine-induced tachycardia by about 30 and almost 80%, respectively (maximum effects) suggesting that α7-nAChRs account for about 40% of the nicotine-induced tachycardia. MAEA (1 µmol/kg) produced an AM 251-insensitive inhibition (by about 40%; maximum effect) of the nicotine-induced tachycardia. Co-administration of MLA and MAEA inhibited the nicotine-induced tachycardia to a similar extent (by about 30%) as each of the drugs alone. The nonadditivity of the effects allows the conclusion that MAEA mediates its inhibition by basically the same receptor as MLA, namely the α7-nAChR, although at an allosteric instead of the orthosteric site.

Different effects of CB1 receptor agonists in ethanol-preferring WHP and ethanol-nonpreferring WLP rats

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WHP alcohol-preferring rats and WLP alcohol-nonpreferring were treated with agonists of cannabinoid CB1 receptor, WIN 55,212-2 (0.5–2 mg/kg, ip) and CP 55,940 (3–30 µg/kg, ip). WIN in the dose of 2 mg/kg and CP 10 and 30 µg/kg induced a significant increase in ethanol intake in WHP rats. On the other hand, both CB1 agonists failed to influence ethanol consumption in the WLP line of rats. The results of the present study suggest that, in contrast to WHP rats, cannabinoid CB1 receptors in the brain are not involved in the regulation of ethanol drinking in WLP line of rats. We suppose that this difference may be related to the mechanism of ethanol preference.
Pharmacokinetic interaction is responsible for inhibition of the anticonvulsive activity of carbamazepine by chronic bupropion in mice

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Bupropion hydrochloride (BUP) is an antidepressant used as a first-line smoking cessation around the world. The main concern associated with BUP therapy is its proconvulsant effect. Recently, it has been demonstrated that BUP can alter the anticonvulsive activity of some antiepileptic drugs. In this work, we show that chronic intraperitoneal (14 days; every 12 h) administration of BUP at subthreshold dose of 5 mg/kg significantly reduces the anticonvulsant activity of phenytoin (DPH) and carbamazepine (CBZ) in maximal electroshock model of convulsions in mice, not affecting however the anticonvulsant activity of valproic acid and phenobarbital. In order to evaluate if pharmacokinetic interaction is responsible for the observed effect of BUP, plasma and brain levels of CBZ and DPH were measured. Samples of blood and brain homogenates were assayed by immunofluorescence, using Abbott TDx analyzer. BUP significantly lowered plasma (from 7.08 to 2.67 µg/ml; p < 0.01) and brain (from 4.03 to 2.16 µg/ml; p < 0.001) levels of CBZ, whereas did not affect plasma (from 1.44 to 1.87 µg/ml) and brain (from 2.30 to 2.06 µg/ml) levels of DPH.

The data showed that the proconvulsant effect of BUP on the anticonvulsant activity of CBZ, but not DPH, results from the pharmacokinetic interaction.

Biotransformation, pharmacokinetics and pharmacodynamics of cinazepam

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A study of metabolism, pharmacokinetics and pharmacodynamics of a novel tranquilizer cinazepam (7-bromo-5(o-chlorophenyl)-1,3-dihydro-3-hemisuccinate-2H-1,4-benzodiazepine-2-one (14C-I, 0.30 Ci/mole) was carried out in mice and rat. In the body, cinazepam undergoes intensive hydrolysis with the formation of 3-hydroxyphenazepam (7-bromo-5(o-chlorophenyl)-1,3-dihydro-3-hydroxy-2H-1, 4-benzodiazepine-2-one (II)), which, in turn, transforms into quinazoline-2-one. Parent compound and II undergo hydroxylation and methoxylation of aromatic nuclei with the subsequent conjugation resulting in the formation of glucuronic and sulfate conjugates. The rate of parallel pathways of cinazepam metabolism varies in different animal species: mostly aromatic hydroxylation is observed in rats, whereas in mice hydrolysis of the ether bond...
with the formation of 3-hydroxylated derivative is predominant. Rapid absorption, distribution and pre-systemic elimination of the drug (I) are noted. We have failed to detect an organ that could serve as a compartment of “slow exchange”. High concentration of the principal active metabolite II has been noted in the brain, the biophase of action, whereas parent drug content there was negligible (5%). Pharmacological studies of cinazepam demonstrated its high anxiolytic, anticonvulsive and hypnotic activity, but lower acute toxicity (higher LD50 values), than classical benzodiazepines such as diazepam, chlorodiazepoxide and nitrazepam. In contrast to many known hypno-sedative drugs, e.g. Nitrazepam or flunitrazepam, cinazepam does not affect normal sleep pattern and prolongs paradoxical sleep.

Kinetics of distribution and excretion of organic derivatives germane in rats

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The kinetic processes of distribution in blood, organs and tissues of rats of potential drug – organic derivatives germane (DG) in an organism has been investigated. It is in parallel studied of kinetics elimination from organism of experimental animals with urine and feces in an interval 0–120 h after single administration. Parameters of formal pharmacokinetics of investigated substances are estimated. DG is characterized by rapid absorption and distribution between the tissues and organs of rats. Our studies of the excretion process of DG showed that during the investigated interval (5 days) from an organism of rats is eliminated with urine and feces about 90% of the doses. Predominant of this or that way of excretion depends on structure of DG. We propose a novel mathematical approach to the analysis of xenobiotic diffusion mass transfer in the “blood tissue” system and their irreversible flow from biosystem on environment (excretion). This variant of diffusion model of pharmacokinetics could be used for estimate of Mean Residence Time (MRT) DG in all biosystem (MRTs):

$$MRT_i = \frac{1}{D} \sum_{i=1}^{m} AUC_i(0-\infty) \cdot V_i$$

where D – dose of the drug (dimensionality – [mass]); AUC_i_0-\infty – values of integrals (areas under the curves) of xenobiotic’s concentration from 0 to \(\infty\) in compartments (i) of volume Vi; B_j_0-\infty and B_j_0-\infty – are quantities of the substance which have eliminated from one of m “j” routes of excretion within the interval 0–t_i to 0–\infty.

The average weighed of values equilibrium tissue (compartments of biosystem)-to-plasma partition ratios (Ks) essentially differ among themselves and assume presence at plasma only 0.6–0.2% of xenobiotic, containing in all biosystem:

$$K_j = \frac{MRT_j}{AUC_{pl}(0-\infty)} \cdot \sum_{i=1}^{m} V_i = \left( \sum_{i=1}^{m} AUC_i(0-\infty) \cdot V_i \right) \left( \sum_{i=1}^{m} AUC_{pl}(0-\infty) \cdot V_i \right)$$

where: AUC_pl_0-\infty values of integrals of xenobiotic’s concentration from 0 to \(\infty\) in plasma.
Impact of changes in P-glycoprotein activity on domperidon pharmacokinetics in rat plasma

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The effect of quinidine (QD) and grapefruit juice (GFJ) extract, P-glycoprotein inhibitors, on the domperidone (DOM) concentration in rat plasma was investigated. DOM, a dopamine D2-receptor antagonist is a substrate for P-glycoprotein. DOM (10 mg/kg) was administered orally 2 hours after GFJ extract (0.2 ml/kg) or QD (25 mg/kg). DOM concentration in plasma samples was determined by HPLC assay with fluorescent detection. The GFJ extract and QD administration significantly increased cmax of DOM by 19% and 36%, respectively and the AUC0-0.25 (area under the concentration-time curve from time zero to 15 min) by 29% and 44%, respectively. In addition, QD significantly increased the DOM AUC0-2 (32%), whereas 19% increase was observed after GFJ extract administration. Thus GFJ and QD significantly influenced DOM rat plasma concentration during the first two hours after DOM administration indicating that interaction takes place during absorption phase.

Genetic polymorphism of CYP2D6 in systemic lupus erythematosus patients

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Systemic lupus erythematosus (SLE) is a complex, multifactor autoimmune disease. SLE is a non-organ specific autoimmune disorder characterized by polyclonal hyperactivity of B lymphocytes and production of pathogenic autoantibodies. In the pathogenesis of this disease, genetic, hormonal and environmental factors are important.

The major route of phase I drug metabolism is oxidation by cytochrome P-450 (CYP) mixed function monoxygenases located within the endoplasmic reticulum. Thirty or more different forms of P-450s have been characterized in humans, each with distinct catalytic specificity and unique regulation. CYP2D6 is one of the first of the well characterized phase I polymorphic drug – metabolizing enzymes and is involved in the oxidation of numerous drugs. CYP2D6 polymorphism has been linked to susceptibility to various diseases including certain cancers and SLE.

In our study we analysed the CYP2D6 alleles and evaluated the genotypes distribution in 45 patients with SLE. The control group consisted of 129 volunteers without any acute and chronic autoimmune disease. The CYP2D6 alleles were identified by polymerase chain reaction fragment length polymorphism (PCR-RFLP) method with DNA extracted from peripheral blood. Among 45 patients with SLE, 20 (44.5%) were homozygous extensive metabolizers (EM), 19 (42.2%) were heterozygous EM, and 6 (13.3%) were homozygous poor metabolizers (PM); whereas in the control group 74 (57.4%) were homozygous EM, 43 (33.3%) were heterozygous EM, and 12 (9.3%) were PM. Comparing the frequency of poor metabolizers genotype in the group of patients with systemic lupus erythematosus and in control group we observed the predominance of poor metabolizers in lupus patients.
Role of cDNA-expressed cytochrome P450 isoforms in \textit{in vitro} cytostatic activity of 5'-chlorophenyl phosphoramidate diesters of 3'-azido-3'-deoxythymidine (AZT)

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Some of nucleoside analogues play an important role in anticancer and antiviral therapy. Azidothymidine (AZT), although originally designed as an antitumor agent, is so far widely used as antiviral inhibitor of HIV-1 reverse transcriptase, that acts as a DNA chain terminator. It is postulated that the mechanism of action of AZT may be associated with its conversion to AZT-triphosphate (AZT-TP), followed by its incorporation by DNA host polymerases. Phosphoramidate pronucleotides seems to be effective for the intracellular delivery of nucleoside 5'-monophosphates (MP) as regards to their \textit{in vitro} cytotoxic activity showed in human tumor cell models. A series of eighteen newly synthesized: 5'-(4-chlorophenyl)- and 5'-(2-chlorophenyl)- phosphoramidate diesters (1DE-18DE) desired as the potentially pronucleotides were studied against KB human tumor cells in tissue culture systems, based on the DR and D, NCI, NIH Bethesda programs. Two mostly active compounds; 5'-(4-chlorophenyl) trifluoroethyl phosphoramidate diester of 3'-azido-3-deoxythymidine (AZT) (4DE) with $ED_{50} = 3.1 \mu g/cm^3$ (0.0057 mol/l × 10$^{-3}$) and 5'-(2-chlorophenyl)ethyl phosphoramidate diester of 3'-azido-3-deoxythymidine (AZT) (11DE) with $ED_{50} = 0.35 \mu g/cm^3$ (0.00072 mol/l × 10$^{-3}$) were qualified for \textit{in vitro} investigation using a mixture of insect cell-expressed human drug metabolizing cytochrome P450 isoforms (CYPs: 1A2, 2C8, 2C9, 2C19, 2D6 and 3A4) model as to be related to their activities in human liver microsomes. That mixture system has been proposed as an experimental model comparable to human liver microsomes for drug biotransformation studies. The results performed with cDNA-expressed cytochrome P450 isoforms showed considerable increase of cytotoxic activity against KB tumor cells for compounds; 4DE and 11DE with their $ED_{50}$ of 0.09 and 0.21 µg/cm$^3$ respectively, in comparison with non-activated control samples. It is proposed that the influence of drug metabolizing CYP450 isoforms plays important role both in chemical reduction of phosphoramidate diesters of AZT with formation to high reactive intermediate of 3'-amino-derivatives metabolites and enhance their \textit{in vitro} cytotoxic activity.

Effect of ABCB1 (MDR1) polymorphism and P-glycoprotein inhibitors on salivary digoxin secretion in congestive heart failure patients

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The aim of the present study was to evaluate effects of ABCB1 (MDR1) gene polymorphism on P-glycoprotein model substrate, i.e. digoxin, salivary secretion. The study was carried out in 77 patients diagnosed with congestive heart failure administered digoxin, who were subdivided into two groups: 1.
co-administered P-glycoprotein inhibitors and 2. without any known P-glycoprotein inhibitors. The ABCB1 2677GA,T and 3435CT polymorphisms were evaluated using PCR-RFLP methods. Steady state digoxin concentrations were measured in blood serum as well as in unstimulated and stimulated saliva using FPIA method. It was found that values of Pearson’s coefficient were significantly higher in patients co-administered P-glycoprotein inhibitors in reference to subjects who were not administered any inhibitor both for stimulated (Pearson’s coefficient $r = 0.832, p < 0.01$) and unstimulated saliva ($r = 0.812, p < 0.01$). Evaluation of the impact of ABCB1 2677GA,T and 3435CT polymorphism on salivary digoxin secretion revealed significant differences in digoxin stimulated saliva/serum ratio between patients stratified by 2677GA,T genotype (TT, TA > GT, GA > GG, $p < 0.01$). The results from the present study suggest that administration of P-glycoprotein inhibitors as well as ABCB1 gene polymorphism may affect salivary digoxin secretion.

Drug-induced malignant hyperthermia in facial/cranial defects

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Malignant hyperthermia is an inherited disorder of skeletal muscle characterized by muscle contracture and hypermetabolic crisis following exposure to halogenated anaesthetics and depolarizing muscle relaxants. Here are the drugs inducing MH crisis:

- Inhaled anesthesia: halothane, isoflurane, enfurane, metoxyflurane, cyclopropane, desflurane, fluoroxene,
- Muscle relaxants: succinylcholine, suxamethonium, decamethonium, galamine, detubocurarine

The disease is often inherited as an autosomal dominant disorder, for which there are at least 6 loci of interest but in a large proportion of cases MH is caused by a mutation of the ryanodine receptor type 1 (RYR1) located at chromosome 19q13.1. RYR1 opens in response to increase in intracellular calcium level mediated by L-type calcium channels, which results in a drastic increase in intracellular calcium levels and muscle contraction. The standard procedure to test persons suspected is a “caffeine-halothane contracture test” (CHCT) but also clinical diagnostic testing of MH susceptibility based on RYR1 mutations is recommended.

Increased risk of malignant hyperthermia was observed in some rare genetic syndromes with facial/cranial defects like Freeman-Sheldon syndrome or Smith-Lemli-Opitz syndrome and in some myopathies such as central core disease (CCD) and others. It still remains unclear what is the mechanism of the association between MH and myopathies and other syndromes mentioned above. Genetic association of RYR1 receptor gene was postulated in central core disease. In other rare syndromes like Smith-Lemli-Opitz syndrome or Freeman-Sheldon syndrome such association was not examined. It is also unknown why MH does not strike during every episode of general anesthesia in MH-susceptible patients and why some patients without genetic defect still can develop MH. The possible explanation of these findings may be the presence of others than described, undiscovered genes involved in MH and/or polyetiological basis of MH. There is a need of future large scale studies in susceptible patients and their families to determine the risk of MH and to find the solution how to avoid MH cases among those not suspected.
Angiotensin II (AII) receptor antagonists are most commonly used in the treatment of hypertension. They are also used as an alternative to angiotensin-converting enzyme (ACE) inhibitors in the management of heart failure or diabetic nephropathy.

The aim of the current study was to evaluate the effects of AII receptor antagonists (losartan and telmisartan), on the protective action of conventional antiepileptics (carbamazepine, phenytoin, valproate and phenobarbital) against maximal electroshock-induced seizures (MES) in mice. Losartan (10, 20 and 50 mg/kg, \textit{ip}) and telmisartan (5, 10 and 30 mg/kg, \textit{ip}) did not influence the threshold for electroconvulsions. In the MES test, both drugs potentiated the protective activity of valproate. Losartan (50 mg/kg) decreased its ED\textsubscript{50} value from 249.8 to 194.6 mg/kg while telmisartan (30 mg/kg) lowered the ED\textsubscript{50} value from 249.8 to 190.6 mg/kg. Telmisartan (30 mg/kg) also enhanced the protective action of carbamazepine, decreasing its ED\textsubscript{50} value from 10.5 to 7.8 mg/kg. Losartan did not influence the anticonvulsive efficacy of carbamazepine. Neither the action of phenytoin nor phenobarbital was affected by losartan or telmisartan.

In conclusion, angiotensin II receptor antagonists can potentiate the anticonvulsant activity of valproate and carbamazepine against MES-induced seizures. This effect may have be significant for patients treated with these drugs.

Analysis of the engagement of I\textsubscript{1} -imidazoline receptors in the studies on imidazoline drugs effect on isolated rat heart atria

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The main sites of action of imidazoline drugs are located in the brain and in peripheral vasculature. Certain imidazolines (clonidine, rilmenidine, moxonidine) exhibit antiarrhythmic effects which are likely to originate from the central nervous system as well as from effects peripheral sites [Bousquet et al.]. Circulatory activity of imidazoline derivatives results mostly from their interaction with \alpha\textsubscript{-}adrenergic and imidazoline receptors. Recently, attention has been payed to the role of imidazolines on physiology of the heart.

In this project the effect of the drugs classified as I\textsubscript{1}-imidazoline agonists (clonidine, rilmenidine, moxonidine and AGN192403) was studied on the rate and amplitude of isolated right and left rat heart atria. The spontaneously beating right atria and the left atria driven electrically were treated with cumulative...
concentrations from $10^{-1}$ to $10^{-3}$ M of the imidazolines studied. The inotropic and chronotropic responses of imidazoline ligands was measured at the presence of fixed concentrations of idazoxan. Effects were calculated as percent of changes of control value of atria rate or amplitude preceding the administration of an agent. Log EC$_{50}$ parameters were also calculated.

Clonidine increases the chronotropic effect on right atria to max. 10.20 ± 1.69% and inotropic effect on left atria to max. 32.07 ± 6.12%. Idazoxan at concentrations $10^{-5}$ and $10^{-3}$ M antagonized the positive chrono- and inotropic effect of clonidine. The presence of idazoxan increases EC$_{50}$ value 100-fold for clonidine chronotropic effects.

Positive inotropic activity of moxonidine and rilmenidine was shown max. 16.24 ± 6.19% and max. 18.27 ± 5.52% respectively. The presence of idazoxan $10^{-3}$ M diminished positive inotropic effect of rilmenidine and partially moxonidine. Log EC$_{50}$ values for moxonidine, rilmenidine were –6.20, and –5.11 respectively. Low EC$_{50}$ value of imidazolines indicated a similar receptor-mediated effect of those compounds. Rilmenidine, moxonidine and idazoxan have no effect on right atria. A weak positive chronotropic activity of AGN192403 was antagonized by idazoxan $10^{-6}$M.

In conclusions: these observations are in agreement with the hypothesis that in inotropic action of imidazoline drugs both α2-adrenergic and I1-imidazoline receptors are engaged. Engagement of imidazoline receptors in chronotropic action remains unclear.

The influence of chokeberry juice on arterial blood pressure
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*Aronia melanocarpa* is a common plant in Eastern Europe and in the North America. The fruit contain a lot of polyphenols especially anthocyanins and caffeic acid and its derivatives in chokeberry are also present in relatively high concentrations. Anthocyanins have antioxidant and anti-inflammatory properties and, therefore, may be potentially used to prevent oxidative stress, frequently associated with cardiovascular diseases.

The aim of the study was to estimate the influence of anthocyanins contained in chokeberry juice on arterial blood pressure, lipid parameters, inflammatory state indices and concentrations of antioxidant vitamins in men with mild hypercholesterolemia.

Healthy men in the number of 58 with the diagnosed mild hypercholesterolemia without pharmacological treatment were involved to the study in 2006. In all men biochemical measurements were carried out 4 times: at the beginning, after 6 weeks of regular chokeberry juice drinking, then after 6 weeks without the juice drinking and after 6 weeks repetition of chokeberry juice drinking. Laboratory tests contained total-, LDL-, and HDL cholesterol and its subfraction in HDL2 and HDL3, triglycerides, lipid peroxides (LPO), C-reactive high sensitivity protein (hsCRP), homocysteine, fibrinogen, glucose and antioxidant vitamins.

Regular chokeberry juice drinking resulted in reduction of total and LDL cholesterol (p < 0.001) as well as triglycerides (p < 0.001) and increased HDL2 cholesterol (p < 0.001) level. Moderate but significant decreases in the serum glucose (p < 0.05), homocysteine (p < 0.001) and fibrinogen (p < 0.01) concentrations were also observed. These beneficial metabolic changes were associated with significant hypotensive effect of chokeberry juice drinking. Our studies showed the real importance of *Aronia melanocarpa* fruit juice drinking on the reduction in the future cardiovascular risk.
Resting heart rate and its change induced by physical training in patients with ischemic heart disease at various age treated with β-blockers

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A goal of the study was an assessment of initial and final resting heartrate (HR) in patients with ischemic heart disease (IHD), younger (group A: 49 patients, 55.5 ± 4.6 years) and elderly (group B: 38 patients, 72.5 ± 4.37 years) treated with β-blockers and subjected to six-month cardiac rehabilitation. All patients underwent acute coronary syndrome episode treated invasively (PTCA) less than 3 months before the rehabilitation and were clinically stable. The two groups were alike as to echocardiographic parameters and (BMI) values. Each of the patients was taking cardioselective β1-blockers (metoprolol or bisoprolol) for at least two months prior to the study. Drug dosage was not modified during the training cycle. Estimation of the β-blockade level was performed using 24-h ECH Holter monitoring and RAMP test. A comparison between the A and B groups concerned initial rHR (min⁻¹): 79.3 ± 8.3 vs. 73.6 ± 8.3 (p < 0.01); after-training rHR: 70.9 ± 7.9 vs. 67.7 ± 8.4 (NS) and delta of rHR (difference between initial and final rHR): –8.4 ± 4.8 vs. –5.9 ± 2.8 (p < 0.01). The statistically significant correlation coefficients between the patients’ age and the mean initial rHR (r = –0.377) and delta of rHR (r = 0.347) were noted.

Conclusion: Reduction of rHR after six-month rehabilitation, resulting from cardiovascular adaptation to regular physical training, was smaller in the older than in the younger IHD patients. The difference seems to be determined by the lower initial rHR in the elderly, which probably was rather caused by physiological vagotonia occurring with age than by therapeutic β-blockade.

Effect of cardiac rehabilitation on triglycerides level in patients with previous myocardial infarction treated with simvastatin

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Effect of supervised long-term cardiac rehabilitation on serum level of triglycerides (TG) and relation between physical training intensity and changes of TG level were analyzed. The study group consisted of 66 patients (age: 59.83 ± 0.96 years) with previous myocardial infarction (MI) treated invasively (PTCA or CABG), who were subjected to six-month cardiac rehabilitation. The control group comprised 32 patients (61.31 ± 1.16 years) after MI and not enrolled to rehabilitation program. The both groups had the same drug regimen (beta-blockers, ACEI, ASA and statins). Simvastatin was taken by all the patients at least for six months before the observation onset in the comparable doses (study group: 19.39 ± 0.96 mg/day vs.
control group: 18.44 ± 1.28 mg/day). In the study period, there were no drug or diet changes. The following results of comparison were obtained: TG initial level (study vs. control group): 121.73 ± 2.48 mg% vs. 124.31 ± 2.98 (NS); delta of TG level after six-month observation (study vs. control group): −15.99 ± 0.6 vs. +2.28 ± 2.65 (p < 0.01). In the study group after rehabilitation, correlation between delta of TG level and both final training intensity, and delta of training intensity was tested. The correlation coefficients were, respectively: r = −0.274 and r = −0.376 (p < 0.01). Conclusion: long-term physical training in MI patients treated with simvastatin induced a decrease of serum TG level, which was significantly more than in the patients without rehabilitation, and this reduction was the more, the more work load was applied during exercise training.

Association between serum adiponectin levels and body mass index in patients with hypertension

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Patients with obesity are susceptible to hypertension. Adipose tissue produces and secretes many bioactive substances conceptualized as adipocytokines, dysregulation in their production is associated with the pathophysiology of obesity-related disorders. Adiponectin is an antiatherogenic and antidiabetic adipocytokine.

The aim of this study was to determine the relationship between adiponectin and essential hypertension. Fasting serum adiponectin concentrations were measured in 32 newly-diagnosed, non-treated patients with mild-to-moderate hypertension, without coexisting illness [18 male (M), 14 female (F)]. The mean age of patients was 43.71 ± 15.28 years. Ambulatory blood pressure measurement (ABPM) was performed in each patient. Both systolic and diastolic blood pressure (SBP, DBP) were assessed on the strength of ABPM. Investigated patients were divided into a subgroup with BMI lesser and greater than 30 mg/kg. The mean SBP was 133.08 ± 12.52 mmHg for all patients and 134.54 ± 12.80 and 132.86 ± 13.02 for M and F respectively. The mean body mass index value was 32.08 ± 6.25 kg/m². Both mean value of SBP and DBP was significantly higher in subgroups with BMI > 30 kg/m² (p < 0.05 and p < 0.01 respectively). The mean adiponectin serum level was 7.45 ± 2.65 ug/ml for all patients and 6.38 ± 2.51 and 8.62 ± 1.62 for M and F respectively. There was statistically significant difference between the groups (p < 0.05). Adiponectin levels were negatively correlated with BMI (p < 0.001) and both SBP (p < 0.001) and DBP (p < 0.01) and positively correlated with age (p < 0.05) for all subjects. There was negative correlation between adiponectin and BMI in M (p < 0.01) as well as with SBP (M p < 0.001; F p < 0.01) and DBP (p < 0.001 for F and M).

Higher serum adiponectin levels are associated with both lower blood pressure and lower BMI values in hypertensive patients.
Analysis of the effect of I2-imidazoline receptors ligands on isolated rat heart atria

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The aim of the study was to determine in vitro the effect of agmatine [(4-aminobutyl)guanidine], BU239 [2-(4,5-dihydrimidazol-2-yl)quinoxaline] and BU224 [2-(4,5-dihydrimidazol-2-yl)quinoline] to the imidazoline binding sites in rat heart. The experiment will consist in the measurements the inotropic and chronotropic effects of the I2-imidazoline receptors ligands on isolated rat heart atria.

The heart was isolated from anaesthetized normotensive Wistar rats. The atria left and right separately were placed in a modified Krebs solution, gassed with carbogen and kept at 37°C. The left atrium was electrically stimulated. Amplitude and rate were measured in response to cumulative concentrations (from 10⁻¹ to 10⁻³ M) of testing ligands in the bath solution. Additionally, effect of several concentrations of idazoxan/phenotolamine on the atria responses to compounds studied was determined. The response to each agent concentration was expressed as percent of 100% control value of atria rate or amplitude preceding the administration of the agent. For the compounds studied log EC₅₀ parameters were calculated.

Agmatine evoked a positive inotropic and chronotropic effect on isolated rat atria. The most marked positive inotropy was observed with maximal effect of 42.0 ± 12.7% and chronotropy of 25.7 ± 4.8%.

Compound BU239 produced positive inotropic activity with maximum effect of 17.4 ± 7.2%. BU239 was found to stimulate the most chronotropic activity of atria (maximum effect was 31.0 ± 9.6%). However, in the presence of idazoxan 10⁻⁷ and 10⁻⁹ M the chronotropic effect of BU239 was reduced. In conclusion, engagement of I2-imidazoline receptors in chronotropic activity of isolated right atria of rat has been postulated.

Impact of nebivolol on level of serum nitric oxide, plasma vWF and exercise stress testing parameters in hypertensive and ischemic heart disease patients

Małgorzata Kobusiak-Prokopowicz¹, Beata Jóździak-Mydlowska¹, Agnieszka Zubkiewicz, Maciej Szymczak, Anna Mysiak, Robert Skalik²

The dysfunction of vascular endothelium precedes the development of atherosclerosis in patients with arterial hypertension. The prevention of endothelial damage may potentially delay the appearance of atherosclerotic plaques in blood vessels. Nebivolol is a very specific β-blocker, which can be characterized by a strong endothelial nitric oxide-mediated vasodilata-
tive effect. Hence, the aim of the study was the assess-
ment of changes in concentrations of serum nitric oxide (NO), plasma von Willebrand factor (vWF) and the selected parameters of electrocardiographic exercise test after 1 month nebivolol treatment in hypertensive patients with stable ischemic heart disease.

Twenty one patients aged from 34 to 82 years (mean age 59.3 ± 12.2 years; 15 females and 6 males) with primary arterial hypertension and ischemic heart
disease, untreated before with β-blocker, entered the study. The blood samples were taken in all the study patients for measurements of concentrations of serum NO, plasma vWF and blood lipids. Following, the electrocardiographic stress test was also performed. Subsequently, nebivolol (5 mg/day) was orally administered in all the examined patients for 4 weeks. The aforementioned biochemical measurements and ecg stress test were repeated after 4 week nebivolol treatment. The significant increase in serum NO concentrations was found in all the investigated patients after nebivolol treatment. The prolongation of exercise time, increase in metabolic equivalent (MET) and decrease in double product were also noted in patients after nebivolol treatment.

The 4 week nebivolol treatment ameliorates heart function and contributes to increase in serum NO concentration.

Effect of selected drugs used as pharmacological doping agents on (3H)glucose uptake in the heart and striped muscle

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Using drugs by sportsmen in order to make their sports results better has been known since the ancient time. Narcotics, anabolics, diuretics, peptide hormones, blood and others agents are on the list of illegal procedures. Glucose is a main source of energy, easy mobilized by tissues. Therefore the aim of the study was to examine effect of selected agents used illegally during sport competitions as a pharmacological doping on (3H)glucose uptake in the heart and striped muscle of rats.

Adult male Wistar rats were injected with ephedrine 2.5 mg/kg, sc, salbutamol 0.05 mg/kg, sc, levo-carnitine 0.1 mg/kg, sc, omnadren (testosterone) 2.0 mg/kg, im or saline 1.0 ml/kg, sc (control) for 14 consecutive days. On the day 14th 60 min after last apply of examined substances all rats were injected with 6-3H-D-glucose 500 Ci/kg, ip. Fifteen minutes later, rats were sacrificed μ (Amersham) and the both ventricular and atrial heart’s and striped muscle were excised. In the samples radioactivity was measured in the scintillation counter. Results were expressed as the disintegrations per minute per 100 mg of wet tissue (DPM/100 mg).

It was shown that ephedrine and salbutamol significantly increased radioactivity in the both ventricular and atrial heart’s and in the striped muscles as compared to the control. In opposite levocarnitine and omnadren decreased radioactivity in examined tissues. By this ephedrine and salbutamol only (but not levocarnitine and testosteron) can increase force in sportsmen probably also via glucose uptake in the heart and striped muscle.

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Interaction of angiotensin-converting enzyme (ACE) inhibitors and conventional antiepileptic drugs in the maximal electroshock-induced seizures in mice

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Angiotensin-converting enzyme (ACE) inhibitors are widely used in the treatment of hypertension and congestive heart failure. They are also used in the management of patients who have had a myocardial infarction and in those with diabetic nephropathy.

The present study examined the effects of ACE inhibitors (enalapril and cilazapril) on the anticonvulsant activity of conventional antiepileptic drugs (carbamazepine, phenytoin, valproate and phenobarbital) against maximal electroshock-induced seizures (MES) in mice. Enalapril (10, 20 and 30 mg/kg, ip) and cilazapril (5, 10 and 20 mg/kg, ip) did not influence the threshold for electroconvulsions. In the MES test, enalapril at the dose of 30 mg/kg potentiated the protective activity of valproate, decreasing its ED₅₀ value from 249.5 to 164.9 mg/kg. In contrast, enalapril at the same dose of 30 mg/kg in combination with carbamazepine, showed proconvulsive efficacy, raising its ED₅₀ from 9.2 to 11.3 mg/kg. Neither the action of phenytoin nor phenobarbital was affected by enalapril. In the present study, cilazapril did not influence the anticonvulsive efficacy of studied antiepileptic drugs.

These results indicate existing interactions between enalapril and two conventional antiepileptic drugs, valproate and carbamazepine, which may have some clinical importance for patients treated with these drugs.
Coordination of colonic and recto-anal motility in rat model

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Motor activity of longitudinal and circular muscles in segments isolated from rat large intestine was recorded using triple organ bath method to display temporal and spatial coordination of motility in colon, rectum and anal canal. Spontaneous high amplitude contractions of both longitudinal and circular muscles of colon and rectum were observed. Longitudinal muscle of colon and rectum responded to electrical stimulation with frequency-dependent contraction. The response of circular muscle of colon consisted of initial frequency-independent relaxation followed by frequency-dependent contraction while the rectal circular muscle responded with contraction indicating a more important reservoir function of the colon than of the rectum. The contractile responses of the longitudinal and circular rectal muscles were considerably more pronounced than the contractions of the colon. The responses appeared synchronously demonstrating co-activation of nervous pathways supplying both muscles of the distal part of the gut. Electrical stimulation elicited biphasic response in internal anal sphincter consisting of contraction followed by relaxation while anal canal responded with contraction. The contractile responses of longitudinal and circular muscles increased from colon to rectum while the relaxation was relatively uniform suggesting an organ-specific predominant contractile function of the smooth muscles in colorectal-anal tube.

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The influence of diabetus mellitus on the development of acute stress-induced gastric lesions in rats

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Diabetes mellitus (DM) is a metabolic disease affecting numerous organ and tissues function, including increased susceptibility of the gastric mucosa to various ulcerogenic stimuli. Knowledge of the role of the metabolic diabetic disturbances in the gastric mucosa resistance against stress is limited.

The aim of the study was to investigate the influence of diabetes on the development of acute stress-induced gastric lesions in rats treated with or without single dose of proton pump inhibitor pantoprazol (PPI-P) or H2 receptors antagonist ranitidine (RA).

DM was induced in male Wistar rats by streptozotocin (STZ), ip, 70 mg/kg. Acute gastric lesions were induced one week after STZ injection by water immersion and restraint stress (WRS). Protective effects of PPI-P or RA were evaluated calculating the damage index (DI) according to Grassi et al. [1991].
In the diabetic rats, a significant increase of stress-induced lesions was observed. DI in STZ-induced diabetic rats and in control group (without DM) was $22.33 \pm 2.63$ and $16.29 \pm 2.46$, respectively. Both, PPI-P and RA, administered 0.5 h before stress, significantly decreased the development of acute stress-induced gastric damages in rats without DM. Protective effects of PPI-P and RA were also observed in STZ-induced diabetic animals (by about 46%), although they were lower as compared to rats with normal glucose blood levels.

DM significantly increases the susceptibility of the gastric mucosa to stress. DM has also deleterious influence on the effectiveness of pantoprazole and ranitidine against acute stress-induced gastric lesions.

Tumor necrosis factor (TNF)-α: role in the development of acute and chronic stress-induced gastric mucosa changes in rats

Teresa Bobkiewicz-Koźłowska, Ewa Korzeniowska-Jasiewicz, Renata Forjasz

Stress is a biological phenomenon, constituting a reaction of the organism to various challenges, both physical and psychological. It leads to a disruption of the balance of the whole organism, including digestive tract. Prolonged duration of deleterious stimuli may lead to formation of diffuse alterations underlying further inflammatory and ulcerative processes.

The aim of the study was: 1) to evaluate to what degree the treatment available for peptic ulcers i.e. antacid sucralfat (SC), H₂ blocker ranitidine (RA), proton-pump inhibitor pantoprazole (PPI-P) protect mucous membrane of the rat stomach during acute and chronic experimental stress; 2) to show whether the protective mechanisms of action of the above-mentioned drugs include blocking the inflammatory process induced by stress.

Acute gastric lesions were induced by water immersion and restraint stress (WRS). Chronic damage to mucous membrane was produced with stress-induced anhedonia as described by Willner [1992]. Mucous membrane damage was evaluated macroscopically calculating the damage index (DI) according to Grassi et al. [1991]. Proinflammatory cytokine TNF-α concentrations in the gastric mucosa were evaluated by ELISA.

The study revealed that treatments available for peptic ulcers administered orally in a single dose before acute stress led to a significant decrease of damage index. Studied agents presented with different levels of protection. SC protected gastric mucosa by 50% and RA by 80%. PPI-P exerted the best protective influence (> 80%). Results of tissue TNF-α correlated with the extent of rat mucous membrane damage both in the studied animals and in the controls. Chronic stress (4 weeks) does not lead to macroscopic changes in the mucous membrane of the stomach but significantly elevates concentrations of tissue TNF-α.

Acute but not chronic stress led to mucous membrane damage. Both acute and chronic stress led to an increase in mucous levels of pro-inflammatory cytokines TNF-α. Among all studied pharmacologic agents, RA and PPI-P exerted the strongest gastroprotective effects in acute experiment. RA and PPI-P, despite different gastroprotective mechanisms of action, prevent the occurrence of stress-related inflammatory changes in the mucous membrane of the stomach due to lowering of tissue TNF-α concentrations. It seems that evaluation of the healing process can be aided by the observation of disappearance of petechiae and monitoring of pro-inflammatory cytokine levels in damaged mucosa.
Dental and skeletal age in children with growth hormone deficiency treated with growth hormone

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Somatotrophic hypofunction (Growth Hormone Deficiency-GHD) is one of basic indications for treatment with growth hormone. One of characteristics of this disease is a delay of bone age in comparison to calendar one. The authors refer to the difference between chronological age and dental and skeletal age in children with growth hormone deficiency depending on duration of treatment with growth hormone.

Twenty five patients with GHD were divided into 2 groups: I – shorter treated ones and II longer treated ones. In children with shorter treatments period delay of skeletal age occurred; biggest in children with shorter substitutional treatments period (group I) – mean about 3 years in contrary to group with longer treatments period (group II), mean over 2 years.

In group I malocclusions represented 66.55%, where of most common were class II malocclusion – 33.33%, then increased overbite and open bites 16.66% of cases. In this group in all subjects dental abnormalities were diagnosed. In group of children treated with growth hormone longer then 1 year (group II) malocclusion represented 86.64%, class II malocclusion 46.66%, scissor – bite and increased overbite 13.33%, furthermore in 6.66% open bite and class III malocclusion were diagnosed. In 33.33% teeth discrepancies were found. In both examined groups increase of G angle on about 0.33% was stated. A longer substitution therapy period, and thus generally longer hormone influence period, intensifies its influence on craniofacial complex. This influence is advantageous and leads to decrease of disproportion in jaw dimensions, thus preventing occurrence of gnathic and malocclusion.

Subcutaneous hematoma after thrombolytic therapy in stroke patients who fall at the disease onset. Report of 4 cases

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Intravenous thrombolysis with recombinant tissue plasminogen activator (rt-PA) is effective therapy for acute ischemic stroke when all treatment criteria are fulfilled. One of the contraindication is significant trauma within 3 months before therapy but it must be remembered that a mild fall at the stroke onset may occur due to sudden hemiparesis or loss of consciousness. This kind of event is not contraindication to thrombolysis and can be easily omitted due to lack of history from the patients (aphasia, impaired con-
sciousness) or from the family. Mild trauma may not leave visible lesions which may appear after thrombolysis and result in subcutaneous or even subfascial hematomas, in some cases required surgical intervention. In the 2nd Department of Neurology, the first thrombolysis in Poland was performed. Up till now more than 130 stroke patients received this kind of therapy. We present four cases of stroke patients who fall at the stroke onsets or were found lying and had no significant signs of trauma at the physical examination at admission. Within several hours subcutaneous hematoma appeared. In these patients no other hemorrhagic complications occurred, including intracerebral hemorrhage what was confirmed in control CT examination.

No reports of subcutaneous hematoma was found in the literature in patients treated with thrombolysis due to heart infarct, probably because they don’t fall during the disease onset. Neurologists applying thrombolysis should pay special account to patient’s history and even slight symptoms of trauma as the complications may be severe, especially in elderly patients.

Marked accumulation of products degradation of kynurenine in saliva and plasma of uremic patients

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Marked increases in toxic metabolites of the L-tryptophan – kynurenine pathway, kynurenine (KYN) and quinolinic acid, were observed in serum and cerebrospinal fluid of both rat and human with renal insufficiency. Recently, we have found increase of concentration of KYN and kynurenic acid (KYNA), but not 3-hydroxykynurenine (3-HKYN) and anthranilic acid (AA) in saliva of hypertensive diabetic patients.

The aim of the study was to estimate the certain kynurenine derivatives in plasma and saliva of uremic patients.

In 19 uremic patients and 19 healthy volunteers the concentration of KYN and its metabolites were estimated by HPLC. In plasma of healthy volunteers the concentration of KYN was 1.6 ± 0.4 mM, of 3-HKYN was 36.5 ± 12.0 nM, of KYNA was 28.8 ± 10.2 nM and of AA was 35.1 ± 4.6 nM. In saliva the concentration of KYN, 3-HKYN, KYNA and AA were 25.9 ± 14.2 mM, 1.2 ± 0.6 mM, 7.3 ± 5.4 mM and 14.0 ± 8.0 mM, respectively. The increase in the concentration of KYN, 3-HKYN, KYNA and AA in plasma and saliva reached 3.1 ± 1.2 mM (p < 0.0001), 467.1 ± 243.7 nM (p < 0.0001), 339.4 ± 189.0 nM (p < 0.0001) and 605.1 ± 280.4 nM (p < 0.0001), respectively.

The marked increased concentration of KYN, 3-HKYN, KYNA and AA in plasma and saliva of uremic patients in comparison to healthy volunteers suggest an altered metabolism of kynurenine in uremia.
Chemopreventive activity of anthocyanins in genotoxically damaged human lymphocyte cultures

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We established that a purified mixture of four anthocyanins isolated from fruit of *Aronia melanocarpa* Elliot (AN) exhibited an antimutagenic activity in the standard cytogenetic tests: AN lowered the frequency of the SCE in human lymphocytes genotoxically-damaged *in vitro* with benzo[a]pyrene (B[a]P; 2.5 µM, 90 min) and lowered the number of cells exhibiting a high number of SCE (cells highly vulnerable to the genotoxic damage with B[a]P). In granulocyte samples, AN lowered the level of the NBT reduction in cells stimulated *in vitro* to generation of oxygen free radicals with phorbol esters or with B[a]P. The presence of AN in lymphocyte cultures (0.78 µM–25.0 µM, 90 min) damaged *in vitro* with B[a]P caused a decrease in the number of cells containing DNA strand breaks in the single cell gel electrophoresis (the comet assay). In genotoxically-damaged cell cultures, AN caused an increase of the apoptotic cells number, as estimated by fluorescent microscope examination after annexinV-FITC/propidium iodide staining. All the results suggest that AN are potent antimutagenic, chemopreventive compounds. We calculated the activity of AN per 1mM of their concentration in the preincubation medium, added the milimolar activity of AN in the above tests and compared the chemopreventive activity when AN were added to the lymphocyte cultures before or after the genotoxic damage with B[a]P. The activity of AN was stronger by about 45% when AN were added to the lymphocyte cultures after the genotoxic damage with B[a]P. It suggests that anthocyanins are able to prevent a cancerous transformation of genotoxically-damaged cells.

The research on interaction of immunosuppressive drugs with ecotoxins in free radicals processes

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Cyclosporine (CsA) and tacrolimus (FK-506) are calcineurine inhibitors with fundamental position in transplantation. Recently they role in oxidative stress is examined. Oxidative stress is connected with transplantation – at first as result of ischemia and reperfusion in transplant organ – secondly as result of inflammation and disturbances in homeostasis in transplant recipients. It was interesting to know whether CsA or FK-506 inhibit or increase free radicals processes. In order to answer the influence on lipids peroxidation, hydroxyl radical generation, ferric reducting ability of plasma and thiol groups were examined. It was also in-
Interesting to know whether common ecotoxin as fluorine or aromatic hydrocarbon give interaction with immunosuppressive drugs in examined processes.

It is proved that several ecotoxins interact with immunological system. Many of them show their toxic effect by free radicals generation. Our investigation pointed out that sodium fluoride in concentrations 3–24 µmol/l increases lipids peroxidation and hydroxyl radical generation. It also attack sulphhydryl groups of proteins.

The investigation was performed on human placental mitochondria and human blood. No toxicological interaction between CsA, FK-506 and sodium fluoride were observed. Cyclosporine only in high concentrations 300 ng/ml slightly increases lipids peroxidation caused by sodium fluoride. The joint effect is connected with hydroxyl radical generation.

Tacrolimus in concentrations 5 ng/ml inhibits lipids peroxidation caused by sodium fluoride, but in concentrations > 10 ng/ml prooxidative effect is more pronounced than antioxidative one. Its joint effect is connected with influence on thiol groups, no hydroxyl radical.

Influence of potential inhibitors of alpha-amanitin hepatic uptake on catalase activity in rats hepatocytes

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Death in Amanita phalloides poisoning is caused by acute failure of the liver which takes up amanitins (AMA) via OATP/Oatp localized in hepatocytes plasma membrane. AMA may cause increase in the levels of ROS because AMA inhibit CAT activity, what contributes to oxidative damage.

The study objective was to examine the role of inhibitors of alpha-amanitin hepatic uptake (Oatp inhibitors) in the protection of CAT in rat hepatocytes. This study was performed on extracorporeally perfused livers of 60 Wistar rats of both sexes, 6 in each group. The animals was divided into 2 control groups: K0 (only perfusion fluid) and K (perfusion fluid + AMA 25 ng/ml) and 8 experimental groups, in which to the perfusion fluid were added AMA (25 ng/ml) and a potential Oatp inhibitor: A (Acetylcysteine, 1 mM), B (Bromosulphite, 11.0 µM), C (Ceftazidime, 1.0 mM), E (β-Estradiol-17β-DGluconide, 320.0 µM), P (Penicillin G, 1.0 mM), R (Rifamycin, 10.0 µM), S (Silibinin, 20.0 µM) and T (Taurocholate sodium, 270 µM). Rat livers were homogenated after 2 hours of extracorporeal perfusion. CAT activity in obtained homogenates was determined.

In rat liver exposed to AMA reduction of hepatocytes CAT activity up to 61.8% (K vs. K0 p ≤ 0.001) was observed. A protective effect of examined inhibitors on CAT activity in rat hepatocytes exposed to AMA in groups A, E, R, S and T was showed. The most effective protection of CAT after addition of Acetylcysteine (A vs. K0 p = NS) or Silibinin (S vs. K0 p = NS) to perfusion fluid was found.
Changes in (CREB) and histone signaling in (COPD)

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It is well established that pro-inflammatory genes are activated in chronic obstructive pulmonary disease (COPD) and asthma, however, glucocorticoids are less effective in COPD than in asthma, indicating that different transcription factors and genes are involved. We assessed the effect of inhaled corticosteroid – budesonide (ICS), with and without theophylline (Th), on signaling pathways related to inflammatory changes in COPD patients. Thirty seven patients with stable disease received for four weeks following courses of therapy: Formoterol (F) alone, F/ICS, and F/ICS/Th bid. Lung function was measured before and after treatment. Cytosol, nuclear extracts and acid extracted histones isolated from induced sputum leukocytes were evaluated for the expression of (CREB) and CREB phosphorylated at Ser 133 (CREB-P), histone deacetylase (HDAC-2), and acetylated histones H3 and H4 (ac-H3 and ac-H4) before and after treatment. We found that F/ICS increased the expression of ac-H3 by 31% (p < 0.001) but decreased both ac-H4 and HDAC-2 expression by 22 and 23% respectively (p < 0.01, p < 001). However F/ICS/Th decreased ac-H3 by 53% (p < 001) in comparison to baseline, and further decreased expression of ac-H4. Expression of CREB was increased in both cytosolic and nuclear fractions by 40% and 24% respectively (p < 0.001, p < 0.01), while CREB-P increased by 50 and 51% (p < 0.01) in both cellular compartments after F/ICS and F/ICS/Th. These findings suggest that ICS/Th treatment may decrease inflammatory molecular signaling pathways activity in COPD. Conversely, activated CREB-related signaling pathways of proinflammatory genes may result in poor response to ICS therapy.

Activity of two newly synthesized fluphenazine analogues in cancer chemoprevention

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Genotoxic agents, both of exogenous and endogenous origin can increase a cancer risk. Since people are constantly exposed to genotoxic agents, it is necessary to search for chemopreventive compounds, able to prevent, stop or reverse the development of cancer. Among the identified chemopreventive agents there are several phenothiazines. Previously we documented that fluphenazine (FPh) exhibited a chemopreventive activity in vitro; however, its use as a chemopreventive drug was limited due to its serious side effects on the central nervous systems. Modification of the chemical structure of parent FPh moiety gave two chemical analogues which were supposed to diminish the psychotropic effects: analogue 1b was more hydrophilic than FPh, and analogue 3f had a bigger molecular weight.

The aim of this study was to compare the chemopreventive activity of FPh and its chemical analogues...
and to define mechanisms of their chemopreventive activity.

The compounds were tested with 8 standard in vivo tests in human lymphocyte cultures genotoxically damaged with benzo[a]pyrene (7.5 µM, 48 h). The tested compounds (concentration range: 0.25 µM –10 µM) lowered the level of the genotoxic damage in lymphocyte cultures (the micronucleus test), decreased the proliferation rate, enhanced apoptosis of genotoxically-damaged lymphocytes and inhibited the transporter function of glycoprotein P.

We found that the chemopreventive effect exhibited by the analogues of FPh was considerably stronger than that of FPh: by 15% in the case of 3f and by about 10% in the case of 1b. Compounds 1b and 3f should be further studied as good candidates for chemopreventive drugs.

**Involvement of putative endothelial cannabinoid receptors in anandamide-induced hypotension and vasodilatory responses in rats**

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Intravenous injection of the endogenous cannabinoid (CB) receptor agonist anandamide (AEA) induces complex cardiovascular changes via cannabinoid CB1, CB2 and vaniloid TRPV1 receptors. In addition, evidence has been accumulating under in vitro, but not in vivo conditions, that AEA relaxes blood vessels via as yet unidentified endothelial CB receptors. (Pacher et al., Pharmacol Rev, 2006).

The aim of our study was to examine whether under in vivo conditions AEA-induced hypotensive and vasodilatory responses are also mediated via endothelial CB receptors. In two groups of rats: anaesthetized and pithed (which were additionally infused with vasoressin), basal diastolic blood pressure (DBP), mesenteric (MBF) and renal (RBF) blood flow were about 65.0 mmHg, 4.4 ml/min and 2.6 ml/min, respectively. In anaesthetized rats, AEA (1.0 mg/kg) decreased DBP, MBF and RBF by about 20, 11 and 12% of the basal value, respectively. All depressor effects were reduced by the antagonist of endothelial CB receptors cannabidiol (CBD; 3 µmol/kg) by 43, 48 and 66%, respectively and by the combined administration of CBD and the CB1 receptor antagonist AM 251 (3 µmol/kg; each) by 85, 80 and 95%, respectively. In pithed rats, AEA (1.0 mg/kg) not affected MBF and RBF but decreased DBP by 15% of the basal value. This effect was diminished both by AM 251 and CBD (3 µmol/kg, each) by 77 and 54%, respectively.

In conclusion, putative endothelial cannabinoid receptors are involved in anandamide-induced decrease in blood pressure and in its vasodilatory effects in renal and mesenteric arteries in rats.
Effects of vigabatrin and phenytoin on the skeletal system in young rats

Barbara Nowińska, Agnieszka Dusiło, Maria Pytlik, Joanna Folwarczna, Leszek Śliwiński, Iłona Kaczmarczyk-Sedlak, Urszula Cegiela, Henryk I. Trzeciak

Long-term administration of anticonvulsant drugs is connected with the risk of impairment of bone remodeling. Contrary to the well-documented unfavourable effect of classical anticonvulsant drugs on bone metabolism, little is known about the effect of the new generation anticonvulsants on bone remodeling.

The aim of the present study was to investigate the effect of vigabatrin, as a representative of new anticonvulsants, on the skeletal system of young rats, in comparison with a conventional drug – phenytoin. The experiments were carried out on 4-week-old male Wistar rats, divided into the control rats, rats receiving vigabatrin (250 mg/kg, po) and rats receiving phenytoin (20 mg/kg, po). The drugs were administered for 28 days. Histomorphometric parameters of the tibia and femur, mechanical properties of the femur, and bone length, diameter, mass, content of mineral substances and calcium were examined.

After administration of phenytoin, the investigated bone parameters did not significantly differ from those obtained in the control rats. Administration of vigabatrin caused the impairment of bone remodeling processes. The observed vigabatrin-induced changes indicate the inhibition of bone formation and intensification of bone resorption.

Influence of statins and free radical scavengers over rabbits platelets on aggregation in the presence of leptin in vitro

Beata Racek-Król, Jacek Petrusewicz

Elevated level of leptin, a hormone which is secreted mostly from adipose tissue, is closely correlated with increase platelets aggregation in mechanism dependent on ADP and it is also connected in directly way with increased risk of incidence of cardiovascular disease.

The aim of the study was comparison degree of inhibition platelets aggregation caused by statins and free radical scavengers with inhibition caused by the same drugs but in the presence of leptin.

Blood samples were withdrawn from the marginal ear veins from the rabbits. It was anticoagulated by 3.8% sodium citrate. The volume ratio of anticoagulant to blood was 1:9. The (PRP) was prepared by routine centrifugation. Platelet aggregation was measured by using an aggregometer (Chrono-Log Co) according to the turbidimetry method of Born. After 2 min. at 37°C preincubation, platelet aggregation was induced by addition of ADP 10 µM and recorded for 6 min. The agents studied were dissolved in 95% ethanol or DMSO. The solution of an agent; 1 µl, fixed concentration, was added to 0.45 ml PRP.

Mevastatin, Lovastatin, Cerivastatin, Pravastatin (10⁻⁴ × 10⁻⁴ g) inhibited platelets aggregation induced by ADP (10 µM) in vitro. Mevastatin (10⁻⁵ g) inhibited platelets aggregation in 28%, Lovastatin (10⁻⁵ g) in 18%, Cerivastatin (10⁻⁵ g) in 15%, Pravastatin (10⁻⁵ g) in 19%. The same effects were observed after using free radical scavengers. Tempol, Tiron, MCP (50 µM, 100 µM, 200 µM) inhibited platelets aggregation: Tempol 3%, 13%, 17%; Tiron 6%, 15%, 22%; MCP 5%, 17%, 16%. Leptin (125 µg) had no effect.
on platelet aggregation. Leptin potentiated the aggregation of platelets induced by ADP. Pravastatin (10⁻⁵ g) and Cerivastatin (10⁻⁵ g) in the presence of Leptin (125 µg) inhibited platelet aggregation in 61% and 55%. Tempol (100 µM) together with Leptin (125 µg) inhibited aggregation in 29%. Tempol (100 µM) inhibited platelet aggregation in 13%.

In summary the results of the present study show that increase inhibition of platelets aggregation induced by ADP, after using Pravastatin, Cerivastatin and Tempol in the presence of leptin (125 µg) in PRP in vitro.

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The action of lansoprazole and pantoprazole in the immunosuppressive test in mice

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Introduction: It was showed that omeprazole may modulate the immunological system in mice. The aim of this study was to investigate the influence of the others proton pump inhibitors – lansoprazole and pantoprazole – on the immunological system.

The study was conducted on adrenalectomized young female Swiss alb mice. The animals were divided in a control group (group A – without adrenalectomy) and 8 experimental groups. Mice in group B received 0.9% saline solution, in group C – prednisolone (5 mg/kg), in groups D, E, and F – lansoprazole at the dose 2.25, 11.25 and 33.75 mg/kg respectively and in groups G, H and J – pantoprazole at the dose of 3, 15 and 45 mg/kg respectively. All tested substances were given for 4 consecutive days. Then the mice were sacrificed, thymuses and spleens were isolated and weighted and thymocytes were counted in the Thoma chamber.

Adrenalectomized mice showed a significant increase in absolute and relative thymus weight and number of thymocytes. Administration of prednisolone prevented these changes. Lansoprazole at all the doses inhibited significantly thymus enlargement (absolute and relative weight) and decreased the number of thymocytes. The immunosuppressive properties of this agent observed in the study were not as strong as those of prednisolone. Pantoprazole did not exhibit this action. No influence of lansoprazole and pantoprazole on spleen absolute weight was noted.

The results suggest that lansoprazole, but not pantoprazole, may modulate immunological system in mice.
Local tolerance examination of human fibroblast growth factor 1 stable mutant in rabbits

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Fibroblast growth factor 1 (FGF-1) exhibit strong angiogenic, osteogenic and tissue-repair properties. It was observed that administration of recombinant growth factors may accelerate wound healing in humans. In the Protein Engineering Laboratory, Institute of Biochemistry and Molecular Biology, University of Wrocław a mutant of FGF-1 with highly increased stability was designed and constructed. Introduced mutations did not affect any of FGF-1 biological activities, increasing, however, its proteolytic resistance and the half life. The drug form of rFGF-1 was prepared in Department of Drug Technology, Wrocław Medical University.

The aim of this study was to investigate the local tolerance of FGF-1 in rabbits what is necessary step before any future clinical trials with this growth factor in humans.

This study was performed on mature alb. rabbits of both sexes according to standards of The European Agency for the Evaluation of Medicinal Products. Animals were divided into 2 groups, 3 rabbits in each: they received rFGF-1 in concentration 1 or 100 µg/ml. The local tolerance after application of FGF-1 on nondamaged skin after single and chronic administration (28 days) was examined. The local test was carried out on nondamaged skin after single dose in occlusive dressing. The local tolerance test after single and chronic administration to the conjunctival sac was also performed.

No changes in skin or conjunctival sac after the applications of FGF-1 in a concentration 1 or 100 µg/mL were observed.

The results suggest that rFGF-1 may be safely applied to the skin and to conjunctival sac. Our study provides a good starting point to further preclinical and clinical studies in wound healing.

The misuse of drugs affecting nervous system: the analysis of expertise reports issued by The Forensic Medicine Department in Wrocław in the period 2002–2006

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When affecting nervous system (ANS) drugs were applied for criminal reasons (homicide), in suicidal attempts, or there is suspicion that medical error could have been involved, such cases are being referred by prosecutor’s offices, courts and police departments to The Forensic Medicine Department where expert physicians analyse these events in cooperation with pharmacologists and toxicologists. The aim of this work was to define ways in which ANS drugs may be misused both by a physician and a patient and to determine the scope of this phenomenon.

The source of data comprised 3177 expertises and investigation raports issued in the period 2002–2005 and the first quarter of 2006 (1Q/2006) by experts
from The Forensic Medicine Department of Wroclaw Medical University. After verification 69 cases were choosen for further analysis.

Documented data were analysed with respect to the misuse type, patient gender, an offender and a drug. The temporal analysis of the phenomenon was also conducted.

Conclusions and observations presented below are based on wholesome analysis of investigated data. 1) Men are involved in majority of misuse cases involving ANS drugs. 2) Criminal misuse of ANS contributed the most to the total number of misuse cases. 3) Ephedrine, tramadol, clonazepam, estasolam, hydroxizine, diazepam, midosalam and temazepam were the most commonly misused ANS drugs. 4) Medical errors have relatively small share in the total number of analysed misuse cases. 5) Many misuse cases involve combining ANS drugs with alkohol. 6) Out of reported deaths 17.24% were attributed to ANS drugs. 7) The control level for dispensation regime concerning ANS drugs should be increased.

Adiponectin and lipid profile in patients with essential hypertension

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Obese individuals have a high incidence of hypertension. Obesity has become the most common nutritional disorder in industrialized countries. A frequent companion of hypertension, dyslipidaemia, diabetes mellitus and other disorders linked to atherosclerotic cardiovascular disease and obesity, especially with visceral fat accumulation, renders it one of the most urgent issue in medicine today. Traditionally regarded as a silent organ that passively stores excess energy, adipose tissue is now considered as an endocrine organ which produces a variety of locally and systemically functioning bioactive molecules, so-called adipocytokines, including leptin, tumor necrosis factor-alpha, plasminogen-activator inhibitor type-1, resistin and adiponectin, that interact with each other and may result in elevated blood pressure. The aim of this study was to determine the relationship between adiponectin plasma levels and lipid profiles in patients with essential hypertension.

Fasting plasma adiponectin (radioimmunoassay method), lipids and lipoproteins concentrations were measured in 34 (19 male, 15 female) newly-diagnosed, never-treated patients with mild-to-moderate essential hypertension without coexisting illness. The mean value of investigated subject’s age was 42.05 ± 16.60 years, body mass index (BMI) was 27.76 ± 4.45 kg/m², WHR 0.89 ± 0.09 cm/cm.

In all 34 patients the mean fasting adiponectin plasma level was 7.28 ± 2.54 mcg/ml, the mean total cholesterol level was 5.98 ± 1.40 mmol/l, LDL cholesterol was 3.69 ± 1.21 mmol/l, triglycerides was 1.55 ± 1.06 mmol/l, HDL cholesterol was 1.58 ± 0.41 mmol/l. The positive correlation between plasma adiponectin concentrations and HDL cholesterol levels (p < 0.001) and negative associations with LDL cholesterol (p < 0.001), triglycerides (p < 0.01) and BMI (p < 0.001) were found. When the investigated subjects were divided into overweight/obese (BMI > 25 kg/m²) and lean (BMI < 25 kg/m²) it showed that the first group has significantly higher adiponectin level than the second one (p < 0.001) and positive correlations between adiponectin levels and HDL cholesterol were found in both cases (p < 0.0001 and p < 0.01 respectively). In the overweight/obese group negative correlations between adiponectin and LDL cholesterol (p < 0.001), triglycerides (p < 0.001) and BMI (p < 0.001) were found. In the lean group analogous associations were less significant for LDL cholesterol and triglycerides (p < 0.01 in both cases) and similar to overweight/obese one for BMI (p < 0.001).
Advantageous lipid profiles are associated with high plasma adiponectin levels in both lean and overweight/obese patients with essential hypertension.

17-β-Estradiol induces hypersensitivity of β1-adrenoceptors in guinea pig left atria

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The effects of female sex hormone estrogen on the heart functions are very complex. Recently obtained data indicate that hormone replacement therapy is not beneficial in prevention of cardiovascular risk at least in some women. Moreover, the mechanisms of estrogen-heart interactions are not clarified sufficiently and require further investigation. We thus undertook this study to investigate the influence of estrogen on the effects of isoprenaline and noradrenaline on guinea pig heart force of contraction.

All experiments were performed on the isolated, electrically driven left atria obtained from males (MC) and females (FC) guinea pigs kept under standard laboratory conditions. The mechanical parameters related to heart contractility, as force of contraction, velocity of contraction and relaxation and duration of contraction and relaxation phases, were measured.

Apart from significantly lower Fc and longer t½ in FC (0.97 ± 0.12 mN, 233 ± 7 ms, respectively) vs. MC (1.66 ± 0.3, 176 ± 18 ms, respectively, n=6, p<0.05) isoprenaline (ISO) and noradrenaline (NOR) (in the presence of prazosine) concentration-response curves were strongly shifted leftward in comparison with males group. Additionally, the maximal effects of NOR was significantly lower in FC (about 40%) than in MC. Application of 17-β-estradiol to males and tamoxifen to females guinea pigs confirmed crucial role of estrogen in observed phenomenon.

Our results indicate that estrogen not only downregulates b1-adrenoceptors, but induces its hypersensitivity to catechol amines, at least in guinea pig left atria.

Antinociceptive effect of aminoacid L-Cysteine in rats

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Cysteine residue is important for function of different receptors. The study performed with SNpys affinity technique revealed the discriminative disulfide-bonding affinity labeling of the three different subtypes of opioid receptors: and Shirasu and Shimohigashi, J Biochem Biophys Methods, 2001. We hypothesized that high doses of cysteine may modulate function of central opioid receptors. In order to confirm this hypothesis we present in this report results of the estimation of antinociceptive effect of intracerebral administration of high doses of cysteine in rats.

The study was performed on adult, female, Wistar rats. A week before experiment rats were implanted with polyethylene cannulas into the right lateral brain
ventricle (icv) under chloralhydrate anaesthesia (300 mg/kg, ip). On the day of experiment unanaes-
thetized rats were icv injected increasing doses of L-
Cysteine at the range 50 to 800 nmols. Moreover
antinociceptive effect of intraperitoneal (ip) injections
of L-Cysteine at doses of 100–400 mg/kg was deter-
mained. Antinociceptive effect was determined by
a tail immersion test. It was found that either icv or ip
administration of L-Cysteine induced significant
antinociceptive effect blocked by pretreatment rats
with naloxone, an unselective antagonist of opioid re-
ceptors. These results indirectly proved that L-
Cysteine modulates activity of central opioid recep-
tors. Moreover obtained results suggest a possible ap-
plying of peripheral administration of L-Cysteine for
analgesic therapy.

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The macromolecular compounds from the popular higher plants
as human blood coagulation inhibitors

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The extracts from selected popular plants from Aster-
aceae and Rosaceae families were prepared and their
anticoagulant activity was measured by APTT and PT
tests. The most promising effect was observed, in
APTT method, for Fragaria vesca and Echinacea
purpurea, which showed the activity closed to the ac-
tivity of 5th International Standard for Unfractionated
Heparin. The structure characterization by IR, PC,
HPLC and colorimetric methods revealed that they
are macromolecular polysaccharide-polyphenolic
products, similar to cell-wall acidic macromolecular
fragments common in the higher plants. The high
content of glucuronic acid and also galacturonic acid,
as well as phenolic fragments seems to be responsible
for the observed anticoagulant activity.

Differential effect of beta-adrenergic blockade on
the mechanical properties of the cancellous and
cortical bone in young rats with
prednisolone-induced osteopenia

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Sympathetic nervous system takes part in the regu-
lation of bone growth and remodeling. Propranolol,
a nonselective beta-adrenergic receptor antagonist,
was reported to counteract bone loss induced by estro-
gen deficiency or mechanical unloading in rodents.
The aim of the present study was to investigate the ef-
effect of propranolol on development of gluco-
corticoid-induced bone damage in young rats.
The experiments were carried out on 6-weeks-old male Wistar rats, divided into 4 groups (n = 8): I – control, II – prednisolone 21-hemisuccinate sodium salt (7 mg/kg, sc), III – propranolol hydrochloride (10 mg/kg, po), IV – prednisolone and propranolol at the above doses. After 28 days of daily drug administration, mechanical properties of the whole femur (extrinsic stiffness, ultimate and breaking load, deformation caused by the applied load) and the femoral neck (load at fracture) were examined. Bone mass, mineral and calcium content, macrometric and histomorphometric parameters were also studied.

In young rats, prednisolone did not significantly affect the mechanical properties of the femur (rats of group II). Only the histomorphometric parameters were impaired in relation to the control rats. Administration of propranolol also did not affect the mechanical properties of the femur, although bone mineralization was improved (rats of group III). Propranolol counteracted the histomorphometric changes induced by prednisolone (rats of group IV) and did not affect the strength of the femoral neck, consisting mainly of the cancellous bone. However, the whole femur measurements, where the load was applied to the diaphysis consisting of the cortical bone, demonstrated that propranolol and prednisolone, administered concurrently, statistically significantly worsened mechanical properties of the cortical bone.

A simple and established method of tissue culture of human gingival fibroblasts for gingival augmentation

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Recent advances in tissue engineering technology implications in its application in different medical fields periodontology. There are some reports of new non-enzymatic methods of isolating human gingival fibroblast for short-time cultivation in vitro, to be used in autologous gingival augmentation. The aim of this study was to obtain a simple and established method of culturing human gingival fibroblasts. The authors developed a recurrent method that can be successfully used in autologous gingival augmentation.

Morphine and diclofenac activities in rat’s estrous cycle

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Previous studies demonstrated that morphine produced greater degree of antinociception in male compared to female. This effect might be attributed to the activation effects of gonadal hormones. The present study determined antinociceptive changes after morphine treatment in female rat’s estrus cycle. It also points to drugs activity changes in animals with hypertension. Two rat’s strains-normotensive WKY (Wistar Kyoto) and SHR (Spontaneously Hypertensive Rats) male and female were investigated. Female’s estrous cycle
phases were cytological determined. The antinociceptive activity of opioid-morphine compared to non opioid – diclofenac was determined by mechanical noxious stimuli. The morphine and diclofenac dosage was 5 mg and 10 mg, respectively.

Statistically significant changes in morphine activity during estrus was observed. There were no antinociceptive differences in estrus cycle after diclofenac administration. SHR are more sensitive to pain stimuli than normotensive rats. Morphine activity is more potent analgesic in normotensive animals as compared to hypertensive. There are no statistically significant changes in diclofenac activity in both considered rat’s strains.

### Antioxidant and antimutagenic activities of polyphenolic preparations from aronia fruit

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We compared the antioxidant and antimutagenic activity of aronia juice (AJ), polyphenolic rich-extract (PF) and a mixture of four anthocyanins (AN) isolated from aronia fruits (Aronia melanocarpa Elliot). The tested preparations were added to the final concentration: 2.5–50 µg/ml (calculated per dry mass) for 60 min. to the exponentially growing V79 (Chinese hamster lung fibroblasts) cell line, previously exposed to hydrogen peroxide (HP; 200 µM in buffered saline, 15 min, 40°C). The intracellular oxidant content was estimated with the H2DCF oxidation-assay, the frequency of apoptotic cells was established by the microscopic examination of cell smears stained with annexin V-FITC/propidium iodide, and the level of DNA strand breaks was estimated by means of the single cell gel electrophoresis (the comet assay). The results proved that procedures of extraction and purification significantly changed biological activities of unprocessed aronia juice: in cell cultures damaged with HP the antioxidant and pro-apoptotic activity was the strongest in the case of AJ, whereas the DNA repair was enhanced, especially in the case of AN preparation. Calculation of the total antimutagenic and antioxidant activity (per 1 µg of dry mass of the tested preparation) in the three tests showed that natural juice of aronia fruit exhibited an antioxidant and antimutagenic activity superior to that of both polyphenolic-rich extract as well as the purified mixture of four anthocyanins.

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### Nicotinamide and its metabolite – N-methylnicotinamide increase skin vascular permeability in rats

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It has been shown that topically applied nicotinamide and its metabolite – N-methylnicotinamide (NMN) might be useful agents for treatment of dermatological disorders such as acne vulgaris or rosacea. In this
study we try to examine if the mechanism of these therapeutic effects.

We used animal model described by Udaka et al. Evans blue (30 mg/kg) was administered intravenously 15 min after intradermal injection of examined compounds and 60 min after application of ointment into Wistar rats anesthetized with Na-pentobarbital (40 mg/kg, ip). To each removed piece of skin containing the dye, 4.0 ml of formamide was added. The results are expressed as the optical density values of the eluates measured at 630 nm in Microtiter® Plate Reader (Dynex Technology, USA) after 72 h incubation at 45°C.

We found dose-dependent increase of vascular permeability in rats treated intradermally by NMN (0.071 ± 0.002, 0.081 ± 0.001, 0.095 ± 0.006 for doses 0.01 mg, 0.1 mg and 1 mg respectively vs. 0.064 ± 0.001 in 0.9% NaCl treated group; p < 0.05, p < 0.001, p < 0.001) and nicotinamide (0.062 ± 0.001, 0.076 ± 0.002, 0.080 ± 0.002 for doses 0.01 mg, 0.1 mg and 1 mg respectively vs. 0.064 ± 0.001 in 0.9% NaCl treated group; ns, p < 0.01, p < 0.001). Interestingly, we observed significantly stronger effect of NMN in comparison to nicotinamide. We also demonstrated increased of vascular permeability in rats treated with 0.5% NMN ointment. Moreover observed effects of nicotinamide and NMN were diminished by both, indomethacin – an inhibitor of prostaglandins synthesis and L-NAME – NO synthase inhibitor.

In conclusion, we provide direct in vivo evidence that nicotinamide and its metabolite – NMN increase vascular permeability in rats, by the mechanism involving NO and prostaglandins.

The use of HPLC assay of plasma paracetamol concentration for the gastric emptying paracetamol test in the dog.

The preliminary study

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The gastric emptying paracetamol test is based on the assumption that paracetamol absorption is minimal in the stomach and rapid in the small intestine. Thus, we decided to elaborate the modified HPLC method of plasma paracetamol concentration measurement in order to elaborate the precise paracetamol test of gastric emptying in the dog.

Two experiments were performed with one-week interval on one dog drinking 100 mg of paracetamol diluted with 50 ml of 0.15 M NaCl. The 1.5 ml blood samples were taken from the leg vein just before and just after paracetamol ingestion and then three aliquots every 10 min, four aliquots every 15 min and finally one aliquot four hours after paracetamol administration. To remove plasma proteins, 250 µl of 1 M HClO4 and 200 µl of potassium phosphate were added to the 250 µl of plasma and samples were centrifuged (16 000 G). Then the samples underwent the chromatographic separation at Waters Alliance 2695 Separations Module with Waters 2996 Photodiode Array Detector and Waters Ascent C18 HPLC column 15 cm × 4.6 mm 5 µm. The mobile phase consisted 0.02 M H₃PO₄ (pH 2.0) and acetonitrile, ratio 85:15. The column flow was 1 ml/min. The column pressure was maintained at 1000 psi, the column temperature was 35°C, and the sample temperature was equal to 25°C. In the first experiment the highest plasma paracetamol concentration (1.13 µg/ml) was found in the sample collected 10 min following paracetamol ingestion. In the course of second experiment the highest plasma paracetamol concentration (0.64 µg/ml) was detected in the sample collected 20 min after paracetamol administration.

These results indicate that the frequency of blood sampling should be increased in the period between 10 and 30 min after intragastric paracetamol administration and that the volume of paracetamol solution used for paracetamol test should be increased.
Effect of thermogenic drugs on core temperature in rats after excessive body cooling in wet conditions

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Exposure to severe cold induces rapid changes in thermoregulatory processes that lead to the increase of heat preservation (behavioral thermogenesis) and production (activation of CNS thermoregulatory centres). Hypothermia develops when heat loss surpasses these two processes. Heat conduction is greatest in wet conditions (e.g., immersion in cold water), therefore hypothermia that occurs in such environment is especially dangerous. Our study was aimed at the evaluation of the possibility of counteracting wet-cold hypothermia by using drugs known to enhance metabolic rate and to induce thermogenesis in thermoneutral conditions.

Experiments were performed on male Wistar rats. In thermoneutral conditions rectal temperature was measured after ip injection of synephrine – agonist of α and β adrenergic receptors (SYN 5, 10, 25, 50 mg/kg), caffeine – phosphodiesterase inhibitor and adenosine receptors antagonist (CAF 40 mg/kg) and BRL-37344-β3 adrenergic receptor agonist (1 mg/kg). SYN did not show thermogenic activity therefore in subsequent experiments CAF and BRL-37344 were used only. Rats swam in water at 12°C for 3 min and their rectal temperature was measured before and after swimming. Tested drugs: CAF and BRL-37344 were injected, ip 30 min before swimming separately or concomitantly.

Although in thermoneutral conditions BRL-37344 and CAF administered both separately or together significantly increased rectal temperature, we did not observe beneficial effect on the drop of rectal temperature after swimming.

The results of our study demonstrated that pre-treatment with thermogenic drugs did not influence hypothermic effect induced by wet conditions.

Calcium antagonists on diazepam withdrawal

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Withdrawal from normal dosage benzodiazepine treatment produced typical anxiety response in animals.

The aim of the study was to investigate the influence of two calcium antagonists on the anxiogenic effects of diazepam withdrawal. After 21 days of treatment with diazepam (2 mg/kg, ip) rats were tested on the 24-, 48-, 72- and 96 h after the last injection/in the elevated plus-maze-test of anxiety. An increase in the percentage number of entries onto the open arms in the elevated plus-maze is interpreted as an anxiolytic response. One-way ANOVA and post-hoc comparison for statistical significance were made.

Compared with control-treated rats, withdrawn animals showed significant decreases in the percent number of entries onto open arms of the plus-maze on the 24th hour [F (1,19) = 5.34; p < 0.03] after the last diazepam injection, indicating an anxiogenic response. On withdrawal calcium channel blockers verapamil (10 mg/kg, po) and diltiazem (15 mg/kg, po) applied during the 21-diazepam treatment and 1 h before testing, significantly reversed the anxiogenic effects after diazepam withdrawal in the elevated plus-maze [F(1,19) = 5.4, p < 0.03; F(1,19) = 6.2; p < 0.03] respectively.

The neurochemical changes underlying in the effect of calcium blockers verapamil and diltiazem are discussed in the light of their possible serotoninergic activity and relationship between serotonin and dopamine neurons. We suggest that chronic treatment with some calcium channel blockers may prove for preventing the development of benzodiazepine withdrawal-induced anxiety.
Effect of bradykinin receptors antagonists on vincristine and streptozotocin induced hyperalgesia in rat model of chemotherapy and diabetic neuropathy

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The role of bradykinin-receptor blockade in development of neuropathies caused by diabetes mellitus and vincristine was examined. Effect of potent and selective B1 receptors antagonist (des Arg10 HOE 140) as well as a specific antagonist of B2 receptors (HOE 140) were investigated. Both agents significantly decreased hyperalgesia caused otherwise by vincristine. In diabetic neuropathy model both agents almost completely suppressed hyperalgesia in first 10 days of study. However from day 11 after administration of streptozotocin action of des Arg10 HOE 140 was significantly weaker than those of HOE 140. Results of the study suggest involvement of both B1 and B2 receptors in transmission of nociceptive stimuli in vincristine-induced as well as diabetic neuropathy model.

Effect of the magnesium ions on analgesic activity of opioids in streptozotocin induced hyperalgesia model

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Diabetic neuropathy (DN) is responsible for one of the most often encountered type of neuropathic pain. DN results from biochemical and functional damage of peripheral nervous system caused by metabolic disorders in course of diabetes. DN accompanying pain is difficult to treat. Classical analgetics, e.g. opioid receptor agonists, possess low activity, therefore other agents, e.g. antidepressants, anticonvulsants or corticosteroids are used. NMDA receptor antagonists have good therapeutic efficacy but also many adverse effects.

Streptozotocin (STZ) produced hyperglycaemia accompanied with chronic increase in nociceptive threshold is considered as a useful model of experimental hyperalgesia. We examined (1) the effect of the opioid receptor agonists on STZ-induced hyperalgesia and (2) effect of the magnesium ions (Mg²⁺) on antinociceptive action of opioid agonists in diabetic neuropathic pain model.

When administered alone opioid agonist like morphine and fentanyl as well as ago-antagonist with potent analgesic activity – buprenorphine had only a little effect on STZ hyperalgesia. However pretreatment with Mg²⁺ markedly enhanced the analgesic activity of all three investigated opioids.

A practical aspect of this phenomenon is discussed.
Fluctuations in endothelial cells and VEGF levels in patients with lymphomas and myelomas do not depend on chemotherapy strategy used

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Level of vascular endothelial growth factor (VEGF) is elevated in various hematological malignancies and adversely associated with prognosis. Circulating endothelial cells are suggested by some authors to be novel markers of angiogenesis.

The aim of the study was to measure circulating endothelial cells (CEC), circulating endothelial precursors (CEP) and activated endothelial cells (aCEC) with 3-color flow cytometry and serum concentrations of VEGF with ELISA in 21 patients with malignant lymphoma (ML) and 20 with multiple myeloma (MM) before and after chemotherapy and to estimate if levels of the studied parameters depend on the type of chemotherapy regimen. In patients with lymphomas CEC, CEP, aCEC and serum VEGF concentrations were significantly higher in comparison with controls. After chemotherapy the significant decrease in CEP numbers and the small although non-significant decrease in CEC and aCEC in patients with ML were observed and it did not depend on the therapeutic regimen that was adopted. In myeloma patients no significant changes in VEGF level nor in the endothelial cells subpopulations were observed and their numbers were similar to obtained from controls.

Results suggest that in patients with lymphomas CEC and CEP, aCEC as well as VEGF may be the markers of malignant process and their elevated levels do not change in short observation period after chemotherapy, simultaneously the measured values did not depend on the type of the therapeutic cycle that was used.

The role of calcium channel blockers in preserving rat liver for transplantation

Małgorzata Trocha, Adam Szeląg

This study aimed at assessment of protective action of nitrendipine and nifedipine on structure and function of rat liver during ischemia and reperfusion time. Rat livers were isolated and preserved for 24 h in HTK solution (4°C) with or without nifedipine or nitrendipine. After preservation the livers were flushed with Ringer solution (supplemented with nitrendipine in one of the groups). Glucose concentration and ALT, AST and LDH activities were measured during perfusion. After the perfusion all the bile produced was collected and livers were histologically examined.

The study showed statistically significant influence of nitrendipine on activity of liver enzymes. Livers both perfused and rinsed with addition of this drug showed the lowest level of enzyme activity and the lowest level of damage in their histological picture among all examined groups. Protective action of nifedipine disappeared after 120 min of perfusion. No statistically significant differences between the groups in
Nitrendipine demonstrates protective influence on function and structure of rat liver preserved for transplantation and maintains its action throughout the perfusion, especially if added not only to perfusion fluid but also to Ringer solution used for rinsing.

Effect of aging process on liver function in extracorporeal rat liver perfusion

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Liver function appears to be well maintained in old age. However, the current state of knowledge about liver aging processes is incomplete. In this study, using extracorporeal liver perfusion model, we evaluated the differences between liver function in young and old rats.

Livers were harvested from groups of young (2 months) and old (12 months) rats and perfused for 2 h with a perfusion fluid. After 10, 30, 60, 90 and 120 min of perfusion, glucose concentration as well as enzyme levels (alanine aminotransferase, aspartate aminotransferase and lactic dehydrogenase) were measured. On completion of perfusion all bile produced was collected.

All measured parameters changed significantly as a function of perfusion time in both groups. Changes in enzyme levels were most evident between 90 and 120 minutes of perfusion. In contrast to old rats, where glucose concentration decreased during all time periods of perfusion, in young rats the glucose concentration increased at the beginning of perfusion. The results suggest that livers obtained from older rats are damaged to a greater extent and are more susceptible to unfavourable conditions during perfusion than livers obtained from younger rats. Also, single measurement of liver enzymes is not enough for complete liver function assessment.

Influence of rilmenidine on intraocular pressure and ocular blood flow in rabbits

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The aim of the investigation was to evaluate ocular hypotensive potential properties of an imidazoline receptor agonist – rilmenidine and, additionally to check its influence on iris microvessels. We compared rilmenidine with an antiglaucoma drug, α2-receptor agonist – brimonidine. An interaction study was also performed, in which rauwolscine and efaroxan (α2 and I1-receptor antagonists respectively) pretreatment was used for evaluation of receptors involved in the ocular action of rilmenidine. The results indicated the agonistic action of rilmenidine on α2-adrenergic receptors and not on imidazoline ones. Rilmenidine occurred to be less vasoconstrictive than brimonidine.
Effect of concurrent administration of alendronate and atorvastatin on histomorphometric parameters of bones in ovariectomized rats

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Estrogen deficiency in women after menopause may lead to development of osteoporosis, hypercholesterolemia and atherosclerosis. The effect of concurrent administration of alendronate and atorvastatin on the processes of bone remodeling in estrogen deficiency has not been studied.

The aim of the present study was to investigate the effect of concurrent administration of alendronate and atorvastatin on histomorphometric parameters of long bones in bilaterally ovariectomized rats.

The experiments were carried out on 3-month-old Wistar rats, divided into 5 groups: I – sham operated control rats, II – ovariectomized control rats, III – ovariectomized rats, which were administered alendronate (3 mg/kg), IV – ovariectomized rats, which were administered atorvastatin (6 mg/kg), V – ovariectomized rats, which were administered alendronate (3 mg/kg) and atorvastatin (6 mg/kg). The drugs were administered to the rats by daily oral gavage (alendronate in the morning, atorvastatin in the afternoon) for 28 days.

Bone mass, mineral and calcium content, macrometric and histomorphometric parameters (endosteal and periosteal transverse growth, width of endosteal and periosteal osteoid, transverse cross-section area of the cortical bone in the diaphysis and of the marrow cavity in the tibia, width of epiphyseal cartilage, width of trabeculae in the epiphysis and metaphysis in the femur) were studied.

Estrogen deficiency resulted in the development of osteopenia with intensified bone resorption in ovariectomized rats. Alendronate caused the preventive effect on the development of the estrogen deficiency-induced osteopenia. Atorvastatin inhibited bone resorption and intensified bone formation and mineralization in ovariectomized rats. Administration of alendronate and atorvastatin together caused synergism in inhibition of development of bilateral ovariectomy-induced osteopenia in rats, which may be the result of concurrent inhibition of different steps in the mevalonate pathway of cholesterol synthesis.

Effects of WIN 55,212-2, a cannabinoid CB1 and CB2 receptor agonist on food intake and body weight in rats with cisplatin induced anorexia

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Appetite reduction as well as body weight loss are significant undesirable effects of cytostatics as they can increase already existing anorexia and cachexia. (THC) as well as nabilone are clinically used to stimulate appetite and weight gain in patients suffering from AIDS or neoplastic diseases. Up to the present, cannabinoids have not been investigated for their ability to counteract anorexia or weight loss during neoplastic diseases chemotherapy.

The aim of the present study was to investigate the influence of WIN 55,212-2, a CB1 and CB2 receptors...
agonist on food intake and body weight in rats with cisplatin induced anorexia. The experiments were carried out on male Wistar rats (180–230 g). WIN 55,212-2 was given at a dose of 3 mg/kg, ip 15 min before or 48 h after cisplatin (6 mg/kg).

Cisplatin reduced food intake at 1 h to 41.7% (p < 0.02), at 2 h to 36.4% (p < 0.05), at 4 h to 30.2% (p < 0.001), at 24 h to 38.9% (p < 0.001) and at 48 h to 30.1% (p < 0.001) vs. control group. WIN 55,212-2 administered before cisplatin eliminated its inhibitory influence on food intake at 1, 2 and 4 h. However, WIN 55,212-2 did not stimulate food total intake during 24 and 48 h. Administered 48 h after cisplatin, WIN 55,212-2 did not counteract cisplatin induced anorexia. Administered before cisplatin, WIN 55,212-2 counteracted body weight loss and the elicited difference was statistically significant in reference to cisplatin (p < 0.05) and statistically insignificant in comparison with the control.

The results show that WIN 55,212-2 administered before cisplatin counteracts anorexia as well as body weight loss caused by this agent. As food intake increase was not long lasting, WIN 55,212-2 protective activity seems to result from other energy intake independent mechanisms.

Phosphodiesterase inhibitors and the production of interleukin-1 by peritoneal macrophages in mice

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The studies were conducted on female Balb/c mice, weighing 20 g (8 weeks of age). Phosphodiesterase inhibitors were administered once or five times at 24 h intervals: aminophylline at a dose of 20 mg/kg, im, milrinone – 1 mg/kg, im; sildenafil – 1 mg/kg, po

The trials in control mice were conducted in parallel.

The level of IL-1 were determined 12, 24 and 72 h after the single dose or 12, 24 and 72 h after the last dose of phosphodiesterase inhibitors.

The production of interleukin-1 in the culture supernatants of peritoneal macrophages stimulated in vitro with LPS from E. coli (055:B5, Sigma) were determined by means of ELISA kit for determination of murine IL-1β (R&D Systems).

The data collected in the study were analysed statistically using a t-test. The differences were considered significant at p < 0.05.

It has been found that a single administration of all tested phosphodiesterase inhibitors enhanced the synthesis and release of IL-1 by peritoneal macrophages 12 h after the drugs administration. The effect of sildenafil was maintained for 24 h. No effect was observed 72 h following the exposure to the drugs.

The administration of selective inhibitors of phosphodiesterase (milrinone or sildenafil) five times at 24 h intervals augmented the synthesis and release of IL-1. This effect was observed only on 24 h following the drugs administration. Aminophylline administered five times did not change the production of IL-1.

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The effect of metronidazole on the sperm properties in pigeon

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Metronidazole is very active antiprotozoal drug which is used successfully in therapy of pigeon trichomoniasis. We demonstrated that metronidazole given at therapeutic doses for the period of 3 months in rats decreased sperm motility and the number of normal and alive spermatozooids. Until now an influence of this agent on spermatogenesis in birds is unknown. The purpose of this study was to examine the effect of metronidazole given at a therapeutic dose on the sperm properties in pigeon.

Sixteen mature male homing pigeons were divided into two groups. The first group was treated with metronidazole at a dose of 50 mg/kg daily for the period of three months and a second one (control) was administered only a vehicle without a drug. Metronidazole was suspended in starch gel and given orally via tube to the throat. Semen collection was conducted via a digital massage before and after 1, 2 and 3 months from the onset of drug administration. Sperm samples were diluted by Lake medium immediately and analysed by HTM IVOS 12.3D system for the estimation of cell concentration and sperm motility parameters. Moreover the nicrosin-eosin stain sperm slides were prepared for a basic morphology examination.

It was ascertained that metronidazole given at a therapeutic dose for the period of 3 months did not influence on the number of normal and alive spermatozooids and sperm motility parameters in pigeons. The results confirmed the hypothesis [Cybulski, 2000] that metronidazol is less toxic for birds as compared to rats by reason of differences in intensity of its reductive bioactivation of the drug in this species.

A subchronic study of the effect of metronidazole on the sperm properties in rats

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It has been reported that metronidazole administered at very high doses in rats (400 mg/kg daily for 30 days) decreased testicular weight, spermatid counts and caused abnormal sperm morphology [Grover et al., 2001]. The aim of this study was to examine the sperm properties in rats treated with lower doses of metronidazole for the longer period of time.

Thirty adult male Wistar rats were used in the trial. The animals divided into 5 equinumerous groups were dosed daily with metronidazole at dose levels 0 (control), 25, 50, 100 i 200 mg/kg for a period of 90 days. The drug suspended in starch gel was given orally via tube to the throat. On day 90 all animals were anaesthetized by pentobarbitone and the testes with epididymes were excised. Afterwards rats were killed by exsanguination and subjected to autopsy. Sperm samples were obtained from the distal cauda epididymis, placed into PBS Dulbacco medium immediately and incubated after technical oil layer at 37°C for 30 min. Computer assisted sperm analysis (HTM IVOS 12.3D) was used for the measurement of sperm concentration and motility parameters. Moreover the nicrosin-eosin stain sperm slides were prepared for a basic morphology examination.

It was stated that metronidazole induced the dose-dependent decrease of mobile \((r = 0.89)\), alive \((r = 0.84)\), and morphologically normal \((r = 0.93)\) cells. The adverse effects were observed even in animals treated...
Cerebrospinal fluid tau protein levels and WISC-R score in children treated for the acute lymphoblastic leukemia

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Event free survival after acute lymphoblastic leukaemia (ALL) now amounts to over 70%, thus allowing observation of a long-term neuropsychological complications.

The aim of this study was to evaluate the level of cerebrospinal fluid tau protein and its correlation with a cognitive test scores.

We examined 38 patients diagnosed with ALL on three points: at the initiation of treatment, during consolidation phase and before initiation of maintenance therapy. The reference group consisted of 22 patients with clinical symptoms of cerebrospinal meningitis. In 19 patients we examined the cognitive functioning in median time of 3.7 years after diagnosis. The mean tau protein level at the diagnosis was 286.8 ± 121.3 pg/ml in the ALL group and 297.6 ± 96.8 in the reference group and showed, at this point, no correlation with initial leukocytosis. Organomegaly and lactate dehydrogenase level. Dynamic date analysis revealed a statistically significant increase in tau protein on the 59-th day of the treatment (401.8 ± 218.67) as compared to its level at the diagnosis and in the reference group. The level of tau protein at the initiation of maintenance therapy was negatively correlated with verbal abilities, measured by intellectual scale. In conclusion, standard ALL chemotherapy can cause neurological disorders, and increased level of tau protein in cerebrospinal fluid may be used as a predictor of future cognitive impairment.

Hypoglycemic activity of aqueous extract of Medicago sativa seeds in diabetic rats

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Although new antidiabetic agents with unique properties have been introduced into the therapy of type 2 diabetic patients in last few years, the search for new antidiabetic agents represents a challenge to the medical profession. *Medicago sativa* (lucerne) has been used the treatment of diabetes but only as a traditional plant.
This study has been undertaken to evaluate the effects of *Medicago sativa* seeds extract on glucose homeostasis in normal and streptozotocin (STZ) diabetic rats.

Neonatally streptozotocin-induced diabetic (n5-STZ) rats (n = 20) treated with aqueous extract of *Medicago sativa* seeds were compared to control (non-diabetic) animals (n = 20) and to diabetic rats receiving glibenclamide treatment for 28 consecutive days. An aqueous extract (1 g/kg) of seeds of *Medicago sativa* or water or glibenclamide (5 mg/kg) were given orally once daily for 4 weeks. Blood glucose levels (fasting, 2 and 4 h after administration) were monitored on 1th, 7th and 28th day of experiment. After termination the blood was collected to estimate 1,5-anhydroglucitol level in plasma – an indicator of acute hyperglycemia. The mean blood glucose (MBG) was calculated.

Plasma glucose levels in 2 h and in 4 h after extract administration were significantly reduced with reference to diabetic controls. Similarly, MBG levels were decreased only in *Medicago sativa*-treated group. 1,5-Anhydroglucitol levels in plasma in *Medicago sativa*-treated group were similar to those observed in non-diabetic rats and were higher in comparison to diabetic controls.

We conclude that chronic treatment with extract of *Medicago sativa* seeds decreases plasma glucose levels in n5-STZ-diabetic rats.

The influence of lovastatine and fenofibrat on serum vitamin A level in rats

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Low fat diet may lead to deficiency of fat-soluble vitamins e.g. A or E. The influence of hipolipemic drugs on the concentration of these vitamins in serum is unknown. The aim of this study was to investigate the influence of chronic treatment with hipolipemic drugs on serum vitamin A level in rats.

This study was performed on 36 mature Wistar rats, fed by standard low fat dry chow. Rats in control group (C) received 1% Tween. Animals in group F received fenofibrate (100 mg/kg) and in group L lovastatin (10 mg/kg) orally by gavage during 6 weeks once a day. Serum vitamin A level was determined after 0, 3 and 6 weeks of experiment.

No significant differences in initial serum vitamin A levels between groups were observed. In group L mean serum vitamin A concentration was significantly higher after 3 and 6 weeks of the experiment (by 54% and 103%, respectively). In group F mean serum vitamin A concentration was significantly higher after 3 and 6 weeks of the study (by 56% and 92%, respectively). No significant changes of serum vitamin A concentration in control group during the experiment were observed. Mean vitamin A level in serum was significantly higher in group F than in group C after 3 weeks of experiment. Mean vitamin A level in serum was significantly higher after 6 weeks in groups F and L than in group C. No significant differences in vitamin A serum concentration between groups L and F were found.

Administration of hipolipemic drugs may increase serum vitamin A level in rats.