Evaluation of antihypertensive effect of L-arginine supplementation in patients with mild hypertension assessed with ambulatory blood pressure monitoring

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Background: Arterial hypertension is one of the most important cardiovascular risk factors, featuring unsatisfactory efficacy of current therapies. Cardiovascular disease paradigm assuming crucial role of endothelial phenotype in shaping the state of circulatory system becomes increasingly dominant and endothelial dysfunction should be treated as avidly as diseases of other organs. Most valued current anti-hypertensive therapies exert positive influence on endothelium due to their pleiotropic effects, yet search for new effective strategies aiming at improving endothelial function is under way. L-arginine trials are part of this quest because this amino acid has been known for its beneficial actions re-establishing the protective properties of the healthy endothelium. Scanty L-arginine studies in hypertension bring inconclusive results.

The aim: The aim of the study was to evaluate antihypertensive efficacy and safety profile of L-arginine during four weeks of oral supplementation to healthy subjects and patients diagnosed with primary mild hypertension.

Material and methods: The study was completed by 54 participants (24 women and 30 men; 38.6 ± 9.36 years old). Ambulatory blood pressure monitoring (ABPM) was used for allotting patients to either healthy control group (19 subjects) or hypertensive treatment group (35 patients). Later patients were randomized to either L-arginine (2 g tid or 4 g tid) or placebo. All participants underwent physical examination and had all basic lab tests and ABPM performed.

Results: Blood pressure (both systolic and diastolic) in ABPM presented with statistically significant lowering after 4 weeks of L-arginine supplementation only in the subgroup of patients treated with 12 g of L-arginine daily with stronger hypotensive effect observed during the day.

Conclusion: The present findings demonstrate strong association between L-arginine supplementation and blood pressure reduction.
Regulation of BDNF expression in trigeminal ganglion neurons by tumor necrosis factor alpha (TNF-α) depends on neuronal activity

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Despite of decades of research, the data pertaining to interactions between the peripheral nervous system and the immune system remain incomplete. Most discoveries have been made in the field of nociceptive signaling. Among pain conditions with the utmost social implications, a large percentage exhibits the inflammatory process as an underlying cause. Here the most prominent are several disorders associated with the trigeminal nerve, including migraine, trigeminal neuralgia, and temporomandibular disorders. The molecular mechanisms of trigeminal nociceptive transmission in general, and chronic trigeminal pain in particular, are almost completely unknown. Our previous studies have shown that release of brain-derived neurotrophic factor (BDNF) from cultured TG neurons is dependent on activity.

To study the effects of an increased neuronal activity on TNF-α-induced changes in BDNF expression in trigeminal ganglion neurons, cultures were electrically stimulated to mimic activity of these neurons in vivo. Results of our experiments demonstrate that the simultaneous exposure of cultures to a 24-h electrical stimulation and TNFα results in an increase in the intracellular BDNF compared to the BDNF content of vehicle-treated control cultures, non-stimulated and stimulated alike.

Our results indicate that electrical stimulation of trigeminal ganglion neurons causes a significant potentiation of the effect of TNF-α on expression of BDNF, pointing to interaction of immune and nervous systems during inflammatory pain.

Effects of lithium on bone mechanical properties in the presence and deficiency of estrogens in rats

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Lithium is a normothymic drug used in the treatment of bipolar affective disorders. Lithium affects numerous cell signaling pathways. It activates the Wnt signal pathway, taking part in the regulation of bone mass. The effect of lithium on the osseous tissue is not well known. The aim of the present study was to investigate the effect of lithium on bone mechanical properties in the presence and deficiency of estrogens in rats.

The experiments were carried out on 3-month-old Wistar rats, divided into 4 groups (n = 8–9 rats per group): I – non-ovariectomized (NOVX) control rats, II – ovariectomized (OVX) control rats, III – NOVX rats which were administered lithium carbonate (NOVX + Li), IV – OVX rats which were administered lithium carbonate (OVX + Li). Lithium carbonate was administered at a dose of 140 mg/kg, po daily for 28 days. The mechanical properties of tibial metaphysis and femoral diaphysis in three-point bending tests and femoral neck in a compression test were assessed (Instron 3342 500N apparatus).

Estrogen deficiency caused development of osteopenia in rats, with worsening of mechanical prop-
properties of the tibia (statistically significant decreases of ultimate load by 41.6% and breaking load by 43.0%), the femoral neck (a significant decrease of the load at fracture by 11.9%), and the femoral diaphysis (significant decreases of ultimate load by 16.2% and breaking load by 17.3%). Lithium significantly improved mechanical properties of the tibial metaphysis ( cancellous bone) and insignificantly improved those of the femoral diaphysis (compact bone) and the femoral neck both in the presence of estrogens and in their deficiency. In tibial metaphyses, lithium increased the ultimate load (by 36.3% in NOVX rats and by 35.1% in OVX rats) and breaking load (by 28.6% in NOVX rats and by 21.4% in OVX rats).

Magnesium ions and opioid analgesia in diabetic-induced neuropathy

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Introduction: Neuropathic pain is a type of pain which is initiated or caused by a primary lesion or dysfunction both in the peripheral and central nervous system. This kind of pain results from metabolic disorders, toxic chemicals or a direct mechanical nerve injury.

The mechanisms underlying neuropathic pain are complex and still not well understood. However, the participation of NMDA receptors in the induction and the subsequent maintenance of neuropathic pain are well confirmed. It was demonstrated that NMDA receptor antagonists can alleviate pain in different experimental neuropathy models. Moreover, NMDA receptor antagonists in combination with opioids have been shown to be more effective than those administered alone. In our study, we demonstrated that magnesium ions, which are physiological NMDA receptors antagonists, unblocked analgesic activity of opioids in diabetic and toxic neuropathy pain models.

Aim: The influence of concomitant administration of magnesium and NMDA receptor antagonist (MK801) on activity of opioids was studied.

Methods: The studies were performed on male Wistar rats. The changes in nociceptive thresholds were determined by using mechanical stimuli (the Randall and Selitto test). Diabetes was induced by intramuscular administration of streptozotocin at a dose of 40 mg/kg of body weight. Streptozotocin produced hyperglycemia accompanied with chronic decrease in nociceptive threshold was considered a useful model of experimental hyperalgesia.

Results: The results of this study confirmed, that morphine, fentanyl and buprenorphine did not alleviate streptozotocin hyperalgesia. Pre-treatment with magnesium markedly enhanced and prolonged the antihyperalgesic activity of opioids. Analgesic activity of opioids was significantly intensified after concomitant administration of MK801 and magnesium ions prior to opioids treatment.

Conclusions: Magnesium ions significantly increase the opioids analgesia in diabetic-induced neuropathy. This effect is probably due to the antagonistic effect that magnesium ions have on NMDA receptors.
Modification of morphine analgesia by antidepressants in diabetic neuropathic pain model

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Introduction: Common side effects of non-treated or ill-treated diabetes lead to diabetic neuropathy. Fighting the neuropathic pain creates a great challenge for contemporary medicine as this kind of pain is resistant to opioidal analgesics. In order to neutralize the neuropathic pain different medications from various pharmaceutical groups are applied i.e. anticonvulsants, antidepressants, local anesthetics and other.

Objectives: Assessing single and long term administration of antidepressants, with various working mechanisms on morphine analgesia, by streptozotocin-induced diabetes model in rats.

Materials and Methods: The research has been conducted on a group of male Wistar rats weighting 250-350g. The model of researched diabetes was achieved by administering streptozotocin (40 mg/kg) in rats intramuscularly on the first day of the experiment. The pain threshold levels were determined by using mechanical stimuli (the Randall and Selitto test). Antinociceptive activity of morphine was assessed after one time and 21 day premedication of antidepressants. The results were later compared to the control group. The density of opioidal receptors of type μi was determined with the use of radioligand binding assay.

Results:
1. After single and multi-dose administration of venlafaxine no significant changes in the density of opioidal receptors type μi have been found in the cerebral cortex in rats with established diabetes.
2. Antidepressants (venlafaxine, fluoxetine) depending on the dose amount, cause the weakening of hyperalgesia in streptozotocin-induced diabetes.
3. One single dose of venlafaxine and fluoxetine produces an intensified effect in morphine’s antinociceptive activity in the model of streptozotocin-induced diabetes.
4. Long term premedication of venlafaxine causes significant blockage of morphine’s antinociceptive activity; as for fluoxetine, it causes slight cancellation of morphine analgesia in the model of streptozotocin-induced diabetes.

Conclusions: The results of tests prove antidepressants from SNRI (Selective Serotonin and Noradrenaline Inhibitor) group (venlafaxine) more effective in neutralising diabetes neuropathy than those belonging to SSRI (Selective Serotonin Reuptake Inhibitor) group (fluoxetine).
Effect of the antiresorptive treatment on development of skeletal changes induced by the combined immunosuppressive treatment with azathioprine and tacrolimus in rats

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The standard immunosuppressive treatment after vascularized organ transplants are calcineurin inhibitors (tacrolimus, cyclosporin), antiproliferative drugs (azathioprine, mycophenolate mofetil) and glucocorticosteroids. Their prolonged use is linked to the risk of the development of osteoporosis. The aim of the present study was to investigate the effectiveness of alendronate administration in the prevention of the development of skeletal changes induced by azathioprine and tacrolimus.

The experiments were carried out on 12-week-old male Wistar rats, divided into following groups (n = 8–10): control rats, rats receiving alendronate (3 mg/kg, po), rats receiving azathioprine (2 mg/kg, po) and tacrolimus (1.5 mg/kg, po), rats receiving azathioprine, tacrolimus and alendronate in the abovementioned doses. The drugs were administered once daily for 28 days. Bone remodeling was assessed based on the evaluation of macrometric and histomorphometric parameters of the tibia and femur, and the mechanical properties of the femur.

Immunosuppression with azathioprine and tacrolimus disturbed bone remodeling. Decreases in bone mass, mass of the bone mineral and calcium content, the osteoid width, the periosteal and endosteal transverse growth, transverse cross-section area of the marrow cavity and the dipaphysis in the tibia, decreasing the width of trabeculae in the femoral epiphysis and metaphysis, as well as the decreasing the extrinsic stiffness, the ultimate and breaking load of the whole femur and the load at fracture of the femoral neck. Alendronate significantly increased bone mass, mass of the bone mineral and calcium content, the width of osteoid, the periosteal and endosteal transverse growth, the width of trabeculae, the extrinsic stiffness, the ultimate and breaking load in the femur in the rats treated with azathioprine and tacrolimus.

In conclusion, alendronate prevented the unfavourable skeletal changes developing in rats with immunosuppression caused by concurrent administration of azathioprine and tacrolimus.

Effects of serotonin and selective serotonin reuptake inhibitors on murine osteoclastogenesis in vitro depend on the estrogen level

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Serotonin has been recently reported to be involved in regulation of bone metabolism in vitro and in vivo. Administration of selective serotonin reuptake inhibitors (SSRIs) may be associated with the increased risk of fracture. Estrogens play a protective role in the skeletal system. Although estrogens have been shown to interact with serotonergic system, in our in vivo study we found that effects of the SSRIs on bone mechanical properties in rats did not depend on the estrogen status [Folwarczna et al., Bone, 44 (Suppl.), 2009].
The aim of the present study was to investigate whether the effects of serotonin and SSRIs on murine osteoclastogenesis in vitro may depend on the estrogen level.

Formation of osteoclasts from neonatal mouse bone marrow cells was stimulated by 1,25-dihydroxy-vitamin D3, added to the culture media the next day after plating, together with serotonin or SSRIs (fluoxetine and fluvoxamine), with or without the addition of estradiol (10⁻⁹ M). Number of osteoclasts, stained for tartrate-resistant acid phosphatase, was determined.

In the absence of additional estradiol, serotonin (10⁻⁷ M–10⁻⁵ M, but not 10⁻⁴ M) and the SSRIs (10⁻⁸ M–10⁻⁵ M, dose-dependently) inhibited osteoclastogenesis. The effect of fluoxetine was stronger than that of fluvoxamine. Addition of estradiol decreased the number of formed osteoclasts (by about 50%). In the presence of the additional estradiol, the inhibitory effect of serotonin on osteoclastogenesis was practically abolished, and the effect of both SSRIs was attenuated in relation to the control cultures with the addition of estradiol.

In conclusion, the effect of serotonin and the SSRIs on murine osteoclastogenesis in vitro strongly depended on the estrogen level. Results of the present study imply that bone marrow cells may be more susceptible to treatments increasing serotonin signaling when the estrogen level is decreased (for example in postmenopausal women).

Effects of high-dose propranolol on development of glucocorticoid-induced osteoporosis in male rats

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Glucocorticoid-induced osteoporosis is the most frequently occurring type of secondary osteoporosis. Antagonists of β-adrenergic receptors are now considered as potential drugs under investigation for osteoporosis. The aim of the present study was to investigate the effects of propranolol, a nonselective β-receptor antagonist, on the skeletal system of mature male rats, and on the development of glucocorticoid-induced bone changes.

The experiments were carried out on 24-week-old male Wistar rats. The effects of prednisolone 21-hemisuccinate sodium salt (7 mg/kg, sc daily) or/and propranolol hydrochloride (10 mg/kg, ip daily) on the skeletal system were studied (n = 8–10 rats per group). Bone turnover markers (serum osteocalcin and tartrate-resistant acid phosphatase 5b), mass, mass of bone mineral in the tibia, femur and L-4 vertebra, bone histomorphometric parameters and mechanical properties of tibial metaphysis and femoral diaphysis (three-point bending tests) and femoral neck (a compression test) were determined.

Prednisolone induced osteoporotic changes (inhibition of both bone formation and resorption), leading to worsening of mechanical properties of the cancellous bone (tibial metaphyses). Propranolol at a high dose did not improve bone parameters, but caused deleterious effects on the skeletal system. Histomorphometric and bone turnover marker measurements demonstrated that propranolol administration decreased bone formation and tended to decrease bone resorption, leading to worsening of the cancellous bone strength. Concurrent administration of propranolol with prednisolone did not counteract the skeletal changes induced by prednisolone, but intensified some of them.

Results of the present study may help understand the unequivocal results of human studies on the effects of β-blockers on the skeletal system. It is possible that the drugs exert biphasic effects on the skeletal system (favorable and deleterious) depending on the dose or individual susceptibility.
Influence of myocardial infarction on the vanilloid TRPV1- and serotonin 5-HT3-receptor-mediated Bezold-Jarisch reflex

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The Bezold-Jarisch reflex (B-JR) is characterized by a sudden bradycardia associated with hypotension, decreased inotropy and coronary vasodilatation. Effects of the B-JR on the circulatory regulation should contribute to the understanding of the pathophysiology underlying ischemic heart diseases including myocardial infarction. The B-JR can be elicited by the activation of serotonin 5-HT3 and vanilloid TRPV1 receptors. Anandamide (AEA), an endogenous agonist of cannabinoid CB1, CB2 and vanilloid TRPV1 receptors, is involved in the pathology of hypotension associated with sepsis, hemorrhage and myocardial infarction [Malinowska et al., J Physiol Pharmacol, 2008]. The aim of the present study was to elucidate the influence of experimental myocardial infarction on the B-JR induced by TRPV1- and 5-HT3-receptor activation.

B-JR was induced by rapid iv injection of AEA (0.2 mg/kg) or the 5-HT3 receptor agonist phenylbiguanide (PBG; 5 µg/kg) to rats anaesthetized with urethane. Both agonists decreased heart rate (HR) by about 7–10% of basal value. They were injected 5 min before induction of myocardial infarction (MI; S1) and 10, 20 and 30 min thereafter (S2-S4). MI was induced by ligation of the left anterior coronary artery. MI potentiated the AEA-induced B-JR by about 105 and 70% 10 and 20 (but not 30) min after MI, respectively. This amplificatory effect of MI was reduced by the TRPV1 receptor antagonist, capsazepine (1 µmol/kg) but not by the CB1 receptor antagonist, rimonabant (0.1 µmol/kg). On the contrary, rimonabant potentiated the amplificatory influence of MI by about 50%. MI also amplified the B-JR elicited by PBG by about 105, 60 and 90% 10, 20 and 30 min after MI, respectively. This amplificatory effect was abolished by the 5-HT3 receptor antagonist, ondansetron (3 µmol/kg).

In conclusion, our results suggest that acute myocardial infarction causes a strong, short-lasting amplification of the Bezold-Jarisch reflex induced via activation of TRPV1 and serotonin 5-HT3 receptors located on sensory vagal nerves in the heart.

Effects of aliskiren on blood pressure and venous thrombosis in renovascular hypertensive 2-kidney 1-clip (2K-1C) rats

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A high number of evidence links the RAS system with thrombosis. For example angiotensin converting enzymes inhibitors (ACE-Is) and angiotensin receptor blockers (ARBs) possess, independent on hemodynamic changes, antithrombotic activity. Aliskiren direct renin inhibitor belongs to a new very attractive class of agents that effectively and specifically inhibit RAS.

The aim of our study was to determine the influence of aliskiren on stasis-induced venous thrombosis in renovascular hypertensive 2-kidney 1 clip (2K-1C) rats.

We compared aliskiren-treated per 10 days 2K-1C rats with vehicle-treated 2K-1C rats and sham-operated controls. Aliskiren did not affect arterial blood pressure in comparison to VEH.
On the other hand, oral administration of aliskiren resulted in dose-dependent decrease of venous thrombus weight \[0.29 \pm 0.3, 0.31 \pm 0.24 \text{ and } 0.13 \pm 0.2 \text{ mg,}\] for doses of 10, 30, 100 mg/kg, respectively, vs. 0.67 \pm 0.58 \text{ mg in the VEH treated group; not significant (ns), ns, } p < 0.01\].

Our preliminary results indicate that aliskiren possess similar to other ACE-Is, not related to hemodynamic changes, the antithrombotic effects. The suggested mechanism of aliskiren action is discussed.

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**Influence of selected angiotensin-converting enzyme inhibitors on alloxan-induced diabetic cataract in rabbits**

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**Background:** Hyperglycemia enhances cataractogenesis. Elevated glucose level is commonly accompanied by arterial hypertension, for which angiotensin-converting enzyme (ACE) inhibitors (ACEIs) are a widely used intervention. ACE inhibitors exert some endothelial pleiotropic actions and can beneficially modulate glucose control and some other metabolic pathways.

**The aim:** The aim of this study was to evaluate the effect of ACEIs on cataract formation in experimental alloxan-induced diabetes in rabbits and assess the role of the reactive function group of the ACEIs in this process.

**Material and Methods:** Two study and two control groups of rabbits were examined. In the study groups and in one of the control groups, diabetes was induced by alloxan. The study groups were assigned to receive captopril or enalapril for six months; the controls received distilled water. Glucose concentration was monitored with a glucometer. A biomicroscope and an ophthalmoscope were used to evaluate lens opacity and cataractogenesis.

**Results:** Six-month administration of ACEIs to rabbits resulted in a delay of diabetic cataractogenesis. The rate of cataract formation was significantly lower in the group treated with captopril than in the enalapril group. A difference in morphology of lens opacity formation between the two study groups was observed.

**Conclusions:** ACEIs delay diabetic cataractogenesis in an experimental animal model. The ACEI functional groups have different influences on the pattern and rate of lens opacity.

**Key words:** Cataract, diabetes, angiotensin-converting enzyme inhibitors, SH-group.
Effects of thalidomide on the skeletal system in young rats
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Thalidomide has been approved for the treatment of multiple myeloma and erythema nodosum leprosum. Clinical trials are currently being conducted for its use in treating inflammatory diseases, various neoplastic diseases, chronic graft-versus-host disease and numerous dermatological conditions, among others, in adult and pediatric patients. The effect on the skeletal system of children has not been recognized.

The aim of the present study was to examine the effect of thalidomide on bone remodeling in young rats.

The experiments were carried out on 5-week-old male rats, divided into 4 groups: I – control, II – thalidomide (15 mg/kg, po), III – thalidomide (30 mg/kg, po), IV – thalidomide (60 mg/kg, po). The drug was administered for 6 weeks.

Bone mass and content of mineral substances were examined in the tibia, femur, and L-4 vertebra. Histomorphometric parameters of the tibia (width of osteoid, diaphysis transverse growth, area of the transverse cross-sectional of the bone marrow cavity and the cortical bone) and the femur (width of trabeculae, width of epiphyseal cartilage) were studied.

The effects of thalidomide on the osseous tissue of young rats depended on the dose.

After administration of thalidomide at a dose of 15 mg/kg, po, the investigated bone parameters did not significantly differ from those obtained in the control rats.

Thalidomide, given at a dose of 30 mg or 60 mg/kg, po, inhibited the processes of cortical bone formation and mineralization (decreases in the bone mass, the width of the periosteal and endosteal osteoid, the transverse cross-sectional area of the cortical bone, and the diaphysis transverse growth were observed). The mineral content in bone also decreased.

Effect of thalidomide on the glucocorticoid-induced histomorphometric parameters changes of bone in rats
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Methods used in the treatment of osteoporosis induced by glucocorticosteroids are not effective enough. There is a need for new drugs which could be useful in counteracting the effects of glucocorticosteroids on bone tissue.

Apart from having written an inglorious chapter in the history of medicine, thalidomide is currently one of the most intensel studied drugs because of its multidimensional activity. Thalidomide has been reported to improve the status of multiple myeloma patients by controlling lytic bone lesions.

The aim of the present study was to investigate the effects of thalidomide on the development of osteoporosis induced by prednisolone (10 mg/kg, po) in 3-month-old male Wistar rats. Thalidomide was administered in a doses of 15 mg and 60 mg/kg, po daily for 3 weeks.

Body mass gain, bone mass in the tibia, femur and L-4 vertebra, histomorphometric parameters of the tibia (width of osteoid, diaphysis transverse growth, area of the transverse cross-sectional of the bone marrow cavity and the cortical bone) and the femur (width of trabeculae, width of epiphyseal cartilage) were studied.

Prednisolone induced osteoporotic skeletal changes in mature male rats (decreases in the bone mass, the
NPYY1 receptors-mediated cardio-respiratory effects of neuropeptide Y in anaesthetized rats

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There is evidence suggesting that neuropeptide Y (NPY), ubiquitous within the neuraxis, contributes to the regulation of breathing. Microinjections of NPY to the dorsal medulla oblongata evoked respiratory and cardiovascular depression [Barraco, Brain Res Bull, 1990; Dunbar, Am J Physiol, 1992]. The infundibular nucleus displayed a high content of NPY in respiratory failure or severe dyspnoea in humans [Corder, Neuroendocrinol, 1990].

The present study was designed to determine the effects of systemic administration of neuropeptide Y on the pattern of breathing and cardiovascular function and to evaluate the contribution of vagal input from below and above the nodose ganglia, and the role of NPYY1 and/or Y2 receptors in these responses.

51 anaesthetized, spontaneously breathing rats were used. Tidal volume was measured at tracheostomy. The timing components of the breathing pattern, arterial blood pressure and heart rate were recorded. Intravenous injection of 100 µg kg⁻¹ of NPY before and after midcervical vagotomy induced immediate slowing down of the respiratory rate and decrease in tidal volume. Supranodose vagotomy eliminated fall in respiratory rate and reduced depression of tidal volume. In all neural states, NPY caused significant hypertension and bradycardia. Blockade of NPYY1 receptors with an intravenous dose of 5 mg/kg of BMS 193885, reduced significantly post-NPY cardio-respiratory effects.

This study has shown that intravenous challenge with neuropeptide Y depresses ventilation due to the decrease in tidal volume and respiratory rate. Hypoventilation is mediated via excitation of NPYY1 receptors outside of the lung vagi. Those present in the nodose ganglia apparently contribute to the timing component of the breathing pattern. The decrease in tidal volume may relay on the brainstem NPYY1 neuronal mechanism. Hypertension and bradycardia occur outside of the vagal pathway and might result from the activation of peripheral vascular or heart NPYY1 receptors.
Transportan peptides modulate phenylephrine vascular action

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Background: Transportan belongs to the family of peptides able to penetrate cell membrane (cell penetrating peptides – CPP). This group of compounds attracted considerations as a potential therapeutic tool for delivery of different substances into the cells. In this study we looked for possible interactions between some of the CPP and phenylephrine vascular action.

Methods: We used isolated rat tail artery and examined the influence of pretreatment by 7 different CPP on concentration-response curve induced by α1 receptor agonist phenylephrine. Peptides were synthesized by the solid phase peptide synthesis (SPPS) with the use of a 9-fluorenylmethoxycarbonyl (Fmoc) strategy.

Results: From 7 different polypeptides: TP10 (transportan-10), [Lys(AAc)13]TP, [Lys(CAc)13]TP, [Lys(GAc)13]TP, [Lys(TAc)13]TP, [Lys(UAc)13]TP, [Lys(Ac)13]TP only TP10, [Lys(AAc)13]TP and [Lys(UAc)13]TP used at concentration 1 µM (the lowest concentration induced significant change in contraction of isolated rat stomach in our pilot study) rendered rat tail artery to be more sensitive to phenylephrine (pD2 and Hill coefficient increased significantly). On the other hand, [Lys(Ac)13]TP decreased efficacy of phenylephrine.

Conclusion: Our results indicate that some polypeptides belonging to the family of CPP can modify affinity of phenylephrine to α1 adrenergic receptors in rat tail artery. Molecular mechanism of these interactions remains to be elucidated.

Rosiglitasone and 15-deoxy-Δ12,14-prostaglandin J2 – PPAR gamma agonists attenuate oxidative stress in chronic experimental CsA nephrotoxicity

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Cyclosporin A (CsA) has played an important role in the improvement of graft survival in patients with solid-organ transplant. However, the clinical use of CsA is limited by main side effect – nephrotoxicity. The renal toxicity of CsA is attributed to reduced blood flow which leads to injury accompanied by excessive generation of reactive oxygen species (ROS). Recent studies indicate that PPAR gamma agonists protect against kidney injury in different experimental pathologic conditions. In this study we tested the effects of the PPAR-gamma agonists: rosiglitasone and 15-deoxy-Δ12,14-prostaglandin J2 on the renal dysfunction and CsA-induced lipid peroxidation.

Methods: Male Wistar rats were treated subcutaneously with CsA (15 mg/kg) alone and in combination with rosiglitasone (8 mg/kg) or 15-deoxy-Δ12,14-prostaglandin J2 (30 µg/kg) for 28 days. 24 h after the last treatment, animals were sacrificed and serum concentration of urea, creatinine and uric acid were determined. Kidney samples were analyzed for MDA + 4HAE (lipid peroxidation products), reduced (GSH) and oxidized (GSGG) glutathione.
Results: Treatment of rats with rosiglitazone and 15-deoxy-Δ12,14-prostaglandin J2 significantly reduced biochemical CsA-induced renal dysfunction. Renal levels of GSH, GSSG, MDA + 4HAE indicated that both PPAR gamma agonists used in this experiment protected animals against CsA-induced oxidative stress in kidney.

Conclusions: PPAR-gamma agonists significantly attenuated oxidative stress induced by CsA in kidney.

Rosiglitazone and 15-deoxy-Δ12,14-prostaglandin J2 – PPAR gamma agonists reduce microscopic changes in experimental chronic CsA nephrotoxicity

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The immunosuppressive drug cyclosporine A (CsA) has been successfully used in several diseases with immunological basis and in transplant patients. Nephrotoxicity is the main side effect of CsA treatment. The histopathology is not specific, includes tubular, vascular, glomerular changes and interstitial fibrosis. Once fibrosis develops, chronic renal failure progresses and is irreversible. Recent studies indicate that PPAR gamma agonists protect against kidney injury in different experimental pathologic conditions. We investigate the effects of the PPAR-gamma agonists: rosiglitazone and 15-deoxy-Δ12,14-prostaglandin J2 on the renal dysfunction and injury caused by CsA.

Methods: Male Wistar rats were treated subcutaneously with CsA (15 mg/kg) alone and in combination with rosiglitazone (8 mg/kg) or 15-deoxy-Δ12,14-prostaglandin J2 (30 µg/kg) for 28 days. 24 h after the last treatment, animals were sacrificed and blood was analyzed for serum urea, creatinine and uric acid levels. Kidney samples were analyzed for histopathological changes.

Results: Treatment of rats with CsA produced a significant increase in biochemical parameters in the serum and marked microscopic changes of cortical proximal tubules, vessels, glomeruli and interstitial fibrosis. Rosiglitazone and 15-deoxy-Δ12,14-prostaglandin J2 significantly protected animals against CsA-induced functional and structural impairment of the kidney.

Conclusions: Cs-A induced nephrotoxicity was significantly attenuated by PPAR-gamma agonists. This study suggests the protective role of PPAR gamma agonists in the pathogenesis of CsA-induced renal functional and histological changes.
Angiotensin-(1–9) enhances arterial thrombosis and platelet aggregation in rats

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Angiotensin (Ang) (1–9) is the renin-angiotensin-system peptide found in the plasma of healthy volunteers and after angiotensin-converting-enzyme (ACE) inhibitors therapy. In vitro experiments proved that Ang-(1–9) may be produced from Ang I by enzymes cleaving one C-terminal aminoacid from Ang I, like ACE type 2 (ACE2).

The aim of our study was to determine the prothrombotic properties of Ang-(1–9) in arterial thrombosis in rat and try to elucidate the role of platelet pathway of Ang II in this process.

Ang-(1–9) infusion resulted in a dose-dependent rise of arterial thrombus weight [0.62 ± 0.05, 1.03 ± 0.12 and 1.08 ± 0.10 mg, for doses of 400, 800 and 1,600 pmol/kg/min, respectively, vs. 0.77 ± 0.08 mg in the VEH treated group; not significant (ns), ns, p < 0.05]. Losartan, a blocker of AT1 receptor for Ang II, abolished the prothrombotic activity of Ang-(1–9).

Losartan, a blocker of AT1 receptor for Ang II, abolished the prothrombotic activity of Ang-(1–9). Ang-(1–9) infused into animals in a dose of 1600 or 400 pmol/kg/min increased collagen induced platelet aggregation (1.45 ± 0.15 for Ang-(1–9) and 1.59 ± 0.16 for Ang II vs. 0.77 ± 0.08 Ω in VEH group; p < 0.05, p < 0.01). Ang-(1–9) also increased platelet aggregation ex vivo (1.81 ± 0.19; 1.87 ± 0.13 and 2.05 ± 0.10 Ω in concentrations of 10–12 M, 10–9 M or 10–6 M, respectively vs. 1.79 ± 0.07 Ω in VEH group; ns, ns, p < 0.05). Additionally, Ang-(1–9) increased the fibrinogen plasma concentration and fibrin generation, without changing PT and APTT parameters.

On the other hand, we detected Ang II and Ang-(1–7) increase after a single iv administration of Ang-(1–9) or only Ang II after incubation of Ang-(1–9) with platelets homogenates.

We conclude that Ang-(1–9) exerts an Ang II-like prothrombotic effect due to the conversion to Ang II in the circulatory system of rats and that platelets are involved in this process.

A comparative study of the effects of three metabolites of furnidipine on hemodynamics and ventricular rhythm disturbances in the in vivo model of ischemia- and reperfusion-induced arrhythmias in rats

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Severe arrhythmias such as ventricular tachycardia (VT) or ventricular fibrillation (VF) are commonly observed in ischemic and certain forms of structural disorders of the heart. Dihydropyridines and other classes of L-type calcium channel blockers are known to protect the heart from ventricular arrhythmias also. Furnidipine (FUR), a dihydropyridine, is oxidatively metabolized in the body and M-2 is known to be such a final metabolite of FUR. We therefore decided to study the influence of three metabolites (M-2, M-3, M-8) of FUR on ischemia-and reperfusion-induced arrhythmias in in vivo rat model to examine their independent activity. Mortality was significantly diminished in M-2 and M-3 treated groups with M-3 preventing animal mortality entirely. All three examined substances significantly reduced the duration and in-
cidence of VF with M-3, once again, completely preventing VF. Moreover, only M-3 significantly decreased the duration of VT but had no influence on their incidence. Through the occlusion and reperfusion phases of the experiment, M-8 was strongly hypotensive as compared to control while such a drastic effect was not seen with M-2 or M-3. Only M-3 was proven to diminish aspartate transaminase (AST) level in the serum. From our observations it might be concluded that among the tested metabolites of furmidipine, M-3 exhibited the most pronounced antiarrhythmic effect while being the most normotensive and therefore was most cardio-protective.

Alpha1-adrenoceptor antagonistic properties of various phenylpiperazine derivatives

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The α1-adrenoceptors subclass has been divided into three subtypes: α1A, α1B and α1D on the basis of pharmacological and cloning studies in various tissues. Due to a relatively low potency of prazosin in functional studies in the lower urinary tract, the existence of fourth α1-adrenoceptor designated α1L (low sensitivity) has been proposed. Since no discrete protein having α1L-adrenoceptor characteristics has been cloned it has been proposed that α1L-adrenoceptors represent a functional state of α1A-adrenoceptors rather than a distinct receptor entity [Eltze, Naunyn Schmiedebergs Arch Pharmacol, 2001]. Analysis of a number of chemical structures of selective α1-adrenoceptor antagonists indicates that majority of active compounds possess arylpiperazine moieties. Various α1-adrenoceptor antagonists can be useful in the treatment of hypertension, benign prostatic hyperplasia (BPH), lower urinary tract symptoms (LUTS) or cardiac arrhythmia [Handzlik, Bioorg Med Chem, 2008; Tait, Bioorg Med Chem Lett, 2005]. In this context, the search for selective α1-adrenoceptor antagonists has been, and still is, an important topic in medicinal chemistry. Thus, we synthesized and tested a series of phenylpiperazine derivatives to find new selective α1-adrenoceptor antagonists.

The compounds were evaluated on their affinity for α1- and α2-adrenoceptors in radioligand binding assays. The antagonistic properties at α1-adrenoceptor subtypes were assessed in functional bioassays. The two most active compounds showed the highest affinity and selectivity for α1-2-adrenoceptors. They turned out to be the competitive antagonists of α1-adrenoceptors with stronger activity at α1D, α1A and α1L and weaker at α1B subtype of α1-adrenoceptors.

The work was supported by grant: K/Z/W/000399.
Association of calpain-10 gene polymorphism and posttransplant diabetes mellitus in kidney transplant patients medicated with tacrolimus

Mateusz Kurzawski1, Krzysztof Dziewanowski2, Karolina Kędzierska3, Wanda Górnik1, Marek Droździk1

New-onset, posttransplant diabetes mellitus (PTDM) has a high incidence after kidney transplantation in patients medicated with tacrolimus, and may adversely affect the patient and graft survival. The pathophysiology of PTDM closely mimics that of type II diabetes mellitus (T2DM). One of possible genetic factors predisposing to PTDM might be polymorphism in calpain-10 gene (CAPN10), previously associated with increased risk of T2DM in general population. Therefore, the present study was aimed at evaluation of CAPN10 gene polymorphisms in PTDM in kidney transplant patients medicated with tacrolimus. A total of 214 nondiabetic kidney transplant patients medicated with tacrolimus (56 patients with PTDM and 158 patients without PTDM were genotyped for the presence of CAPN10 gene variants (SNP-43: rs3792267:G>A, SNP-19: rs3842570 ins/del and SNP-63: rs5030952:C>T) using PCR-based assays. Frequency of SNP-63 minor allele was slightly increased in PTDM patients (P = 0.056), and an association of SNP-63 heterozygosity and the risk of PTDM (odds ratios (OR) = 2.45, P = 0.023) was observed. An increased odds for PTDM development in patients carrying 1-1-2 haplotype (rs3792267:G-rs3842570:ins-rs5030952:T) compared to noncarriers was also noted (OR = 2.35, P = 0.026). Patients’ higher body mass index and SNP-63 minor T allele carrier status were identified as independent PTDM risk factors, confirmed by multivariate regression analysis. This is the first study of CAPN10 polymorphism in relation to PTDM risk. However, the application of SNP-63 (rs5030952:C>T) as a marker of PTDM should be verified by further independent studies.

Pharmacokinetic interactions of enrofloxacin during absorption from the gastrointestinal tract in turkeys

Włodzimierz Markiewicz1, Tomasz Maślanka1, Piotr Jakubowski1, Tomasz Grabowski2, Justyna Pawęska1, Małgorzata Siemianowska1, Jerzy Jaroszewski1

Pharmakokinetic interaction of fluoroquinolones during absorption from the gastrointestinal tract in animals are not well known. Therefore, the aim of the present study was to determine the influence of food and calcium with magnesium on bioavailability of enrofloxacin from the gastrointestinal tract of 4-weeks old turkeys. Enrofloxacin (10 mg/kg b.w.) was administered as follow: Group I (n = 8) – intravenously, Group II (n = 8) – per os with food, Group III (n = 8) – per os without food, Group IV (n = 8) – per os with calcium (14 mg/kg b.w.) and magnesium (0.38 mg/kg b.w.). Blood samples were collected before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12 i 24 h after drug administration (in Group I, additionaly at 5, 15 i 45 min), centri-
Enrofloxacin bioavailability after treatment with food was about 51% lower (p < 0.05) as compared to that found after intravenous administration. In fasted animals, bioavailability was very good and almost complete, while after co-administration with calcium and magnesium, absolute bioavailability was reduced by 32% (p < 0.05). Some pharmacokinetic parameters in all experimental groups are presented in table (see above). The results obtained indicate, that food and ions of calcium and magnesium significantly reduced absorption of enrofloxacin from the gastrointestinal tract in turkeys. Therefore, for effective treatment, their co-administration should be avoided.

### Influence of morin-5’-sulfonic acid sodium salt (NaMSA) on cyclophosphamide-induced changes in oxido-redox status in rat liver

Anna Merwid-Ląd¹, Ma³gorzata Trocha¹, Tomasz Sozañski¹, Jan Magdalan¹, Ewa Chlebda¹, Dorota Ksi¹dzyna¹, Ma³gorzata Pieœniewska¹, Lidia Fereniec-Go³êbiewska¹, Maria Kopacz², Anna KuŸniar², Dorota Nowak², Adam Szel¹g¹

#### Background:
Cyclophosphamide (CPX) is an anti-cancer drug with strong immunosuppressive properties which makes this drug useful in the treatment of some autoimmune diseases. The long-term use of CPX is limited by its adverse effects which, at least partly, are connected to the induction of oxidative stress in tissues. Flavonoids are potent antioxidants and may prevent from some adverse effects induced by CPX.

#### Aim:
The aim was to evaluate effect of morin-5’-sulfonic acid sodium salt (NaMSA) – a water-soluble derivative of natural morin on oxido-redox status in rat liver.

#### Materials and Methods:
Experiment was carried out on Wistar rats of both sexes (203.9 g ± 18.6 g) ar-

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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intravenous injection</th>
<th>Per os + fed</th>
<th>Per os + fasted</th>
<th>Per os + Ca and Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2\text{el}}$ (h)</td>
<td>5.08 ± 0.57</td>
<td>15.23 ± 3.71*</td>
<td>5.76 ± 0.76</td>
<td>4.10 ± 0.21</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>0.08 ± 0.0</td>
<td>5.06 ± 1.09*</td>
<td>2.19 ± 0.34*</td>
<td>1.65 ± 0.27*</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td>2.60 ± 0.39</td>
<td>0.44 ± 0.03*</td>
<td>1.87 ± 0.22</td>
<td>1.02 ± 0.06*</td>
</tr>
<tr>
<td>AUC$_{\text{t0-15}}$ (mg/l h)</td>
<td>10.75 ± 1.72</td>
<td>5.29 ± 0.46*</td>
<td>13.09 ± 1.37</td>
<td>7.27 ± 0.38*</td>
</tr>
<tr>
<td>$C_{\text{t0-15}}$ (l/h kg)</td>
<td>1.12 ± 0.22</td>
<td>1.39 ± 0.17</td>
<td>0.80 ± 0.10</td>
<td>1.38 ± 0.07</td>
</tr>
<tr>
<td>MRT$_{\text{t0-15}}$ (h)</td>
<td>6.04 ± 0.46</td>
<td>23.53 ± 5.30*</td>
<td>7.67 ± 0.57</td>
<td>6.87 ± 0.23</td>
</tr>
<tr>
<td>F (%)</td>
<td>–</td>
<td>49.14 ± 4.30</td>
<td>99.0 ± 3.0</td>
<td>67.58 ± 6.30</td>
</tr>
</tbody>
</table>

$t_{1/2\text{el}}$ – half-life time in elimination phase; $t_{\text{max}}$ – time to reach maximum concentration; $C_{\text{max}}$ – maximum plasma concentration; AUC$_{\text{t0-15}}$ – area under concentration-time curve; $C_{\text{t0-15}}$ – observed total body clearance; MRT$_{\text{t0-15}}$ – mean residence time; F – bioavailability; * p < 0.05
Effects of morin-5′-sulfonic acid sodium salt (NaMSA) on cyclophosphamide-induced toxicity in rats

Anna Merwid-Ląd¹, Ma³gorzata Trocha¹, Tomasz Sozański¹, Jan Magdalan¹, Ewa Chlebda¹, Dorota Książęza¹, Ma³gorzata Pieśniewska¹, Lidia Fereniec-Go³êbiewska¹, Maria Kopacz², Anna KuŸniar², Dorota Nowak², Adam Szeląg¹

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²Department of Inorganic and Analytical Chemistry, University of Technology, Powstańców Warszawy 6, 35-499 Rzeszów, Poland

Background: Cyclophosphamide (CPX) is an anticancer drug with strong immunosuppressive properties that makes this drug useful in long-term treatment of some autoimmune diseases. Such use of CPX is limited by its adverse effects. There is a great need to find substances with low toxicity which may reverse toxic effects of CPX. It was shown that flavonoids may prevent some adverse effects induced by anticancer drugs.

Aim: The aim was to evaluate the effect of morin-5′-sulfonic acid sodium salt (NaMSA) – a water-soluble derivative of natural morin as a protective factor in CPX-induced toxicity.

Materials and Methods: Experiment was carried out on Wistar rats of both sexes (203.9 g ± 18.6 g) arranged in 3 experimental groups (N = 12): group C – receiving 0.9% saline; group CX – receiving CPX (15 mg/kg); group MCX – receiving CPX (15 mg/kg) and NaMSA (100 mg/kg). All substances were given intragastrically for 10 days. Rats were observed and weighed every day. On 11th day animals were sacrificed and blood morphology and biochemical parameters (total protein, glucose, creatinine, urea, alanine aminotransferase (ALT), asparagine aminotransferase (AST), gammaglutamyltranspeptidase (GGTP) and amylase) were evaluated.

Results: CPX significantly inhibited body weight gain and this action was not reversed by NaMSA. CPX also caused significant decrease in plasma protein level, and significantly increased creatinine level, and GGTP activity. Only in case of creatinine NaMSA partly reversed the action of CPX.

In blood CPX significantly decreased white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB) and platelets (PLT) levels but NaMSA partly reversed only RBC and HGB levels, without impact on WBC or PLT.

Conclusions: In this study only slight impact of NaMSA on CPX-induced toxicity was observed. From many studied parameters only GGTP activity, RBC and HGB levels were partly restored by NaMSA. In contrast to earlier works water-soluble derivative of morin (NaMSA) is not so potent antidote in CPX-induced toxicity.

ranged in 3 experimental groups (N = 12): group C – receiving 0.9% saline; group CX – receiving CPX (15 mg/kg) and 0.9% saline; group MCX – receiving CPX (15 mg/kg) and NaMSA (100 mg/kg). All substances were given intragastrically for 10 days. On 11th day animals were sacrificed half of the liver was homogenized and samples were prepared for spectrophotometric determination of lipid peroxides (LPO), superoxide dismutase (SOD) and glutathione (GSH).

Results: CPX induced marked oxidative stress in liver. Significant increase in LPO level together with significant decrease in SOD activity were observed. LPO level was completely reversed by the treatment with NaMSA, and SOD activity was partly restored by this compound. No significant changes were seen in GSH levels between all groups. However, CPX caused slight decrease in GSH concentration.

Conclusions: Observed changes in oxidative stress parameters (LPO and SOD) were similar to these shown in our earlier works [Magdalan J at al., Pharmacol Rep, 2007, suppl. 1; Chlebda E et al.; Exp Tox Pathol, 2010] in which we documented potent action of NaMSA on cadmium induced changes in oxidoredox status in mice liver. Also in this study NaMSA decreased CPX-induced elevation in LPO level, but influence on SOD activity was not so strong.
Angiotensin-(1–9) enhances stasis-induced venous thrombosis in rat due to the impairment of fibrinolysis

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Background: Since ACE-2 enzyme alternatively converting angiotensin (Ang) I into Ang-(1–9) and Ang II into Ang-(1–7) has been discovered there is still lack of data concerning the properties of Ang-(1–9). A high number of evidence links the RAS system with thrombosis.

Methods: We have investigated the influence of Ang-(1–9) on stasis-induced venous thrombosis in Wistar rats. The role of the AT1 receptor, Ang-(1–9) metabolites (measured by HPLC) and coagulation and fibrinolytic parameters (measured in coagulometer or by ELISA) in the mechanisms of action was determined.

Results: Ang-(1–9) infusion resulted in increase in BP (14 ± 3 mmHg in comparison to –1 ± 3 mmHg in VEH treated animals; p < 0.01) and a rise of venous thrombus weight (0.61 ± 0.12, 0.83 ± 0.19 and 0.86 ± 0.14 mg, for doses of 400, 800 and 1600 pmol/kg/min, respectively vs. 0.45 ± 0.14 mg in VEH treated group; ns, ns, p < 0.05). LOS administered together with Ang-(1–9) reversed the increase of thrombus weight caused by this peptide. We observed significant increase of active PAI-I levels (1.98 ± 0.38 for Ang-(1–9) vs. 0.95 ± 0.13 ng/ml in VEH treated group; p < 0.05) and fibrin generation (72.61 ± 4.91 for Ang-(1–9) vs. 46.18 ± 7.70% in VEH treated group, p < 0.05), whereas t-PA levels decreased (0.1 ± 0.02 for Ang-(1–9) vs. 0.22 ± 0.05 ng/ml in VEH treated group; p < 0.05) in rat plasma. Ang-(1–9) failed to influence APTT, PT and fibrinogen (Fg) plasma concentrations. We also found similar increased concentrations of Ang II and Ang-(1–7), and small amounts of Ang-(1–9) in rat plasma one hour after start of infusion of Ang-(1–9).

Conclusions: Ang-(1–9) via the AT1 receptor enhances thrombosis development. The prothrombotic effect of Ang-(1–9) may partially depend on fibrinolysis impairment accompanied by accelerated fibrin formation. Elevated Ang II plasma level during intravenous infusion of Ang-(1–9) indicates that the action of Ang-(1–9) could be mediated by Ang II.

Evaluation of antinociceptive and anti-inflammatory properties of novel [3,4-d]piridazine derivatives in animal models of experimental pain and in in vitro tests

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The current therapies for pain remain unsatisfactory and actually used drugs have a number of limitations. There is a need for newer analgesic and anti-inflammatory drugs that have greater efficacy, better adverse effect profiles and reduced potential for toxicity. It has been reported previously that some of [3,4-d]piridazine derivatives produced analgesic effects in mice. Most of them showed strong analgesic properties and was non-toxic [Śladowska H, Boll Chim Farmac, 2004]. Previous studies from our laboratory have consistently shown potent activity of compounds TZ-68 and TZ-70 in “writhing syndrome” test that suggests peripheral mechanism of their action. The aim of this study was to carry out further pharmacological investigations which can lead to explanation of the exact mechanism of action of investi-
 gated [3,4-d]piridazine derivatives. Investigated compounds were administrated by intraperitoneal injection and their activity was assayed in several models of pain. The cumulative response time of nociceptive behaviors induced by an intraplantar formalin injection was reduced by TZ-68 and TZ-70 treatment during the both 1st and 2nd phases in a dose-dependent manner. In addition these compounds attenuated oedema in the carrageenan-inducted inflammation and showed an antinociceptive effect in a dose-dependent manner in capsaicin- and glutamate-induced nociception. Moreover, they , concentration – dependently, shifted the histamine response to the right without depression of the maximal response, indicating a competitive interaction with histamine receptors present in the guinea-pig ileum. Inhibitory effect of investigated compounds on PDE in biochemical assay was also shown.

Therefore, these results indicate that [3,4-d]piridazine derivatives show an antinociceptive property in various pain models and may possess the therapeutic potential to treat the inflammatory pain. They have differential analgesic and anti-inflammatory mechanism involving histamine H1 receptor antagonism and PDE inhibition but others mechanisms can not be excluded.

Testing conception of engagement of imidazoline receptors in effects of imidazoline agents on isolated rat heart atria

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Circulatory activity of imidazoline drugs results mostly from their interaction with α-adrenergic and/or imidazoline receptors located in brain and in peripheral vasculature. Recently, attention has been payed to the role of imidazolines in physiology of the heart. However, no systematic comparative studies were performed nowadays regarding the activity of a representative series of imidazoline drugs towards imidazoline receptors in isolated rat heart atria preparations.

The aim of the study was to determine inotropic and chronotropic effects of I1/I2 imidazoline receptor ligands: rilmenidine, moxonidine, clonidine, 2-BFI, BU239, BU224 and benazoline on isolated rat heart atria. The spontaneously beating right atria and the electrically driven left atria were treated with cumulative concentrations of the agents studied. The inotropic and chronotropic responses of imidazolines were measured additionally at the presence of various concentrations of imidazoline receptor (idazoxan) or α-adrenergic (yohimbine/phenotamine) receptor antagonists. Those effects were calculated as per cent of changes of the control value of atria rate or amplitude preceding the administration of each agent studied. The –log EC50 parameters were also calculated.

The positive inotropic effect was evoked with the rank order of potency: clonidine > benazoline > BU224 > BU239 > rilmenidine > moxonidine. Those effects were diminished by idazoxan. BU224, BU239, clonidine and benazoline exerted positive chronotropic effects, antagonized by idazoxan. 2-BFI weakly diminished the rate of beating atria, but moxonidine and rilmenidine had no effect. In conclusion, imidazoline receptors of I1 subtype may be involved in inotropic reaction of the agents studied, but this effect depends mainly on the α2-adrenergic receptors. Engagement of I2 imidazoline receptors, along with the α2-adrenergic ones, in chronotropic activity of isolated right atria of rat has been also demonstrated.
Intercorrelations of selected parameters in working heart study after non-treated myocardial infarction in rats and their potential diagnostic and therapeutic relevance for humans

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Background: The relationship between hemodynamic functions and the cardiac remodeling after the myocardial infarction (MI) has been intensively studied, but the characterization of the post-MI heart state is often narrowed down to insufficient number of parameters and by not taking them into account jointly. Here, based on experiment with non-treated post-MI rat hearts, we demonstrate, that in order to determine the actual heart state and to make a better prognosis, it is indispensable to measure a number of hemodynamical parameters over an extended period of time and even more importantly, to intercorrelate them.

Methods and Results: The analysis presented here is based on the experiment carried out on 78 Sprague-Dawley rats subjected to an experimentally stimulated heart infarction induced by permanent occlusion of the left coronary descending artery for 2, 4, 6, 11, 21, 28, 35 or 70 days and followed up by the measurements of 10 hemodynamic parameters: the heart rate, left atrial pressure, systolic and diastolic pressures, coronary and aortic flows, maximum rate of aortic pressure increase and decrease, pO₂, pCO₂, pH measured in pulmonary effluent, and myocardial oxygen consumption (MVO₂) calculated according to Zander formula. The statistical analysis allows us to point out the three-stage evolution of the post-MI hearts. We show that the intercorrelations (intercorrelation diagrams) included in the analysis of each stage allow not only to determine the actual stage of the post-MI heart evolution, but also to predict its future development.

Conclusions: The analysis of the data allows us to single out three stages of the post-MI heart time evolution and to quantify each of the stages. The intercorrelation diagrams provide the post-MI evolution of hemodynamic parameters (like history “paths”). We believe that these results may have an importance also for humans. It may help also in the ambulatory practice e.g. in monitoring of relevant heart parameters (measured in a non-invasive manner), as well as predicting the heart state development, specifying a proper diagnosis, and finally, in singling out an optimal treatment.

The influence of long-term administration of Curcuma longa extract on antioxidant processes as well as on motor activity in aged rats

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The Curcuma longa extract, derived from a rhizome of a traditional medicinal plant, contains curcuminoids of a well-known antioxidant activity. The aim of this work was to summarize the effects of its chronic pre-treatment in aged Wistar male rats on antioxidant processes in their organs. The livers, kidneys, heart and skeletal muscles were isolated from 24-month old animals fed for 2-month either with a standard chow
diet (Con) or with chow enriched with the plant extract (the dose of 10 and 50 mg of curcumin powder per kg b.w.; C10 and C50 groups respectively). The levels of reduced glutathione (GSH), glutathione s-transferase (GST) and catalase (CAT) were estimated. The increased concentration of GSH in the livers (F(2,20) = 7.46; p < 0.004) and the kidneys (F(2,20) = 9.45; p < 0.001) in comparison to control animals was seen (GSH: C50 14.47 ± 1.19 nmol/mg vs. Con 10.51 ± 0.77, p < 0.01, NK test in the livers and C10 5.06 ± 0.35, p < 0.01, NK test; C50 5.6 ± 0.32, p < 0.001 vs. Con 3.72 ± 0.21 in kidneys) but not in the heart and the skeletal muscles. The enhanced levels of GST were observed in the kidneys (F(2,20) = 5.5; p < 0.01; C50 0.32 ± 0.028, p < 0.01, NK test vs. Con 0.21 ± 0.016) and in the skeletal muscles (F(2,20) = 27.82; p < 0.0000; C10 0.098 ± 0.003 µmol/min/mg, p < 0.0001, NK test, C50 0.094 ± 0.002, p < 0.0001 vs. Con 0.075 ± 0.003). The increased level of CAT was seen only in the skeletal muscles in both extract-fed groups of rats in comparison to control group (F(2,20) = 4.99; p < 0.01; CAT: C10 13.07 ± 1.19 U/mg, p < 0.01, NK test, C50 11.04 ± 1.3, p < 0.05 vs. Con 7.49 ± 1.21). No differences of any checked parameters were found in the heart muscle. The motor activity of animals estimated both in the Morris water maze and in the Hole-board remained unchanged. The elevated GST levels may improve the defence against electrophile toxic compounds. The increased GSH concentrations, a main nonenzymatic antioxidant and GST cofactor, and elevated CAT activity in the tissues of plant extract-treated aged rats may be related to the greater ability of scavenging the free oxygen radicals by curcuminoids. Concluding, chronic *Curcuma longa* extract administration may influence antioxidant processes in livers, kidneys and skeletal muscles of aged rats.

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**Azathioprine induces disorders of bone formation in rats**

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Azathioprine (AZA) is a potent immunosuppressive drug, used in the prophylaxis of transplant rejections and in the treatment of autoimmune diseases. Immunosuppressive drugs are known to disturb bone remodeling. The effect of AZA on the skeletal system has not been reported so far.

The aim of the present study was to investigate the effect of AZA on the rat skeletal system, and the effect of alendronate, a drug used in the treatment of osteoporosis, on development of skeletal changes induced by AZA.

The experiments were carried out on 12-week-old male Wistar rats, divided into following groups (n = 8): control rats, rats receiving AZA (4 mg/kg, po), rats receiving alendronate (3 mg/kg, po), rats receiving AZA and alendronate in the abovementioned doses. The drugs were administered once daily for 28 days. Bone remodeling was assessed based on the evaluation of macroscopic and histomorphometric parameters of the tibia and femur, and the mechanical properties of the femur.

AZA disturbed bone remodeling, reducing the osteoid width, the periosteal and endosteal transverse growth, transverse cross-section area of the marrow cavity and the diaphysis in the tibia, decreasing the width of trabeculae in the femoral epiphysis and metaphysis, bone mass, mass of the bone mineral and calcium content, as well as the decreasing the ultimate and breaking load of the whole femur and the load at fracture of the femoral neck. Alendronate counteracted the development of the skeletal changes induced by AZA. Alendronate increased bone mass, mass of the bone mineral and calcium content, the width of osteoid, the periosteal and endosteal transverse growth, the width of trabeculae, the extrinsic stiffness, and the ultimate and breaking load in the femur.

In conclusion, AZA at a dose of 4 mg/kg for 28 days induced bone remodeling disorders, inhibiting bone formation. Alendronate prevented the development of skeletal changes caused by AZA administration, inhibiting bone resorption.
The influence of novel phenylpiperazine xanthone derivatives on the isolated rat’s aorta

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The newly xanthone derivatives with methoxyphenylpiperazine moiety have been examined for their antagonistic properties at the vascular α1-adrenoceptor within functional bioassays on the isolated rat’s aorta. Based on the radioligand binding assays results, which confirmed affinities for α1-adrenoceptors at nanomolar range, a group of compounds has been selected. Previous studies have shown strong antiarrhythmic and hypotensive activity of these compounds.

The α1-adrenoceptor antagonistic activity was assessed by inhibition of phenylephrine-induced contractions. The investigated compounds, concentration-dependently, shifted the phenylephrine response to the right. For two compounds, designated as MH-94 and MH-99 a Schild slope did not differ significantly from unity, indicating a competitive interaction with the α1-adrenoceptors, so pA2 value was determined. The results of antagonistic potency for the tested compounds were in agreement with radioligand binding results.

Blocking effect of pentapeptide Any-GS on MAS MT I-induced analgesia in rats

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MAS MT I is a decapeptide isolated from brain of larval Manduca sexta. It belongs to myotropins and produces cardiostimulatory effect on semi-isolated heart of Tenebrio molitor. Moreover it was found that MAS MT I after intracerebroventricular injection exerts antinociceptive effect in rats and this effect was not blocked by naloxone, an opioid antagonist [Ryka-czewska-Czerwińska M, Pesticides, 2008].

Any-GS is a pentapeptide isolated from wild silkworm Antheraea yamamai and acts as a growth suppressor. Synthetic peptide suppresses activity of rat hepatoma cells. But biological study on Tenebrio molitor proved that Any-GS shows a strong cardioinhibitory effect. It was postulated that Any-GS could be antagonist of proctolin, the first synthetic insect peptide which was found as antinociceptive agent in rats.

The aim of this study was to explore influence of Any-GS on pain perception in rats and then check potential antagonistic activity of Any-GS on antinociceptive effect MAS MT I.

The experiments were performed on adult female rats. A week before experiment animals were subjected to the procedure of implantation polyethylene cannulas into the right lateral ventricle (intracerebroventricularly, icv) using the technique described in our previous papers. On a day of experiment investigated peptides were injected directly icv. Effect of Any-GS on pain perception was determined by a tail immersion test. It was found that Any-GS exerts biphasic effect: firstly antinociceptive effect and later 150 min after injection hyperalgesic effect, maximally at a dose of 50 nmol. Moreover it was discovered that
Any-GS blocks antinociceptive effect of MAS MT I, but only at the dose of 50 nmol.

A possible mechanism of both peptides: MAS MT I and Any-GS is discussed. We suppose that Any-GS may be useful tool substance to block biological effects of some insect neuropeptides.

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**Effect of insect octapeptide leucopyrokinin (LPK) and its active analog [2–8]-leucopyrokinin ([2–8]-LPK) on plasma level of cortisol in rats**

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It was found in our laboratory that two synthetic insect neuropeptides: octapeptide leucopyrokinin (LPK) (p-Glu–Thr–Ser–Phe–Thr–Pro–Arg–LeuNH₂) and its active analog [2–8]-leucopyrokinin ([2–8]-LPK) applied into the lateral brain ventricle (icv) elicited antinociceptive effect in rats. This effect is mediated by central opioid receptors. Moreover it was demonstrated that icv administration of [125]I-labeled LPK resulted in a high accumulation this peptide in hypothalamus and in hippocampus and the highest accumulation in adrenals of rats. This finding prompted us to evaluate effect of both peptides: LPK and [2–8]-LPK on cortisol releasing into blood of rats.

The study was performed on male Wistar rats, which a week before experiment were implanted with polyethylene cannula into the lateral brain ventricle, using the same technique as in our previous reports. On the day of experiment dissolved peptides were injected icv: LPK at the doses of 5 and 20 nmol and [2–8]-LPK at the doses of 5 or 100 nmol. Moreover these both peptides were injected in a dose of 100 mmol/kg, ip. Separate groups were injected with: [D-Ala⁵]-[2–8]-LPK in a dose of 5 nmol icv 15 min before icv injection and naloxone hydrochloride 1 mg/kg, ip 30 min before ip injection of [2–8]-LPK. After 120 min rats were anaesthetized with chloralhydrate (300 mg/kg, ip) and the blood was taken by heart puncture. In blood plasma was determined level of cortisol by ELISA method.

It was found that LPK at the dose of 20 nmol icv, and [2–8]-LPK at the dose of 5 nmol icv significantly increased cortisol levels in rat serum. This effect was blocked either by prior icv administration of [D-Ala⁵]-[2–8]-LPK, or by pretreatment rats with naloxone hydrochloride at the dose of 1 mg/kg, ip.

Obtained results indicate that cortisol releasing effect of both insect neuropeptides: LPK or [2–8]-LPK is mediated by opioid system. Thus the results of present study displayed a novel, noteworthy hormonal effect of this insect peptide.

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Effect of ramipril on bone mechanical properties in the presence and deficiency of estrogens in rats

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Estrogen deficiency in postmenopausal women can lead to the development of osteoporosis and arterial hypertension. The most frequently used drugs in the treatment of hypertension are angiotensin converting enzyme inhibitors. Angiotensin II was demonstrated to stimulate resorptive activity of osteoclasts in vitro. The effects of angiotensin converting enzyme inhibitors on the osseous tissue are not well known.

The aim of the present study was to investigate the effect of ramipril on bone mechanical properties in the presence and deficiency of estrogens in rats.

The experiments were carried out on 3-month-old Wistar rats, divided into 4 groups (n = 8–10 rats per group): I – non-ovariectomized (NOVX) control rats, II – ovariectomized (OVX) control rats, III – NOVX rats which were administered ramipril (NOVX + R), IV – OVX rats which were administered ramipril (OVX + R). Ramipril was administered at a dose of 5 mg/kg, po daily for 28 days. The mechanical properties of tibial metaphysis and femoral diaphysis in three-point bending tests and femoral neck in a compression test were assessed (Instron 3342 500N apparatus).

Estrogen deficiency caused development of osteopenia in rats, with worsening of mechanical properties of the tibia (statistically significant decreases of ultimate load by 44.5% and breaking load by 47.1%), the femoral neck (a significant decrease of the load at fracture by 21.1%), and the femoral diaphysis (significant decreases of ultimate load by 8.9% and breaking load by 13.3%). Ramipril worsened bone mechanical properties. This effect in rats with the presence of estrogens was stronger than in the ovariectomized rats. In NOVX rats, ramipril significantly worsened the mechanical properties of the cancellous bone (tibial metaphysis), causing significant decreases in the ultimate load by 22.9% and breaking load by 21.0%, and the compact bone (femoral diaphysis), causing decreases in the ultimate load by 10.6% and breaking load by 10.5%, as well as the femoral neck (a significant decrease of the load at fracture by 13.5%).

The influence of neonatal capsaicin-induced C-fibres injury on analgesic activity of gamma-butyrolactone derivative in mice

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Capsaicin, a pungent ingredient of chili peppers is a potent stimulant of unmyelinated afferent C-fibres’ injury when administered to mice or rats in early neonatal period (between 2nd and 5th day of life). For this reason it serves as a pharmacological tool to establish the role of those fibres in the nociceptive transmission. In the following experiment the analgesic activity of 3-[4-[3-(trifluromethyl)-phenyl]-piperazin-1-yl]-di-hydrofuran-2-on (LPP1) was investigated in the writhing test and since acids are strong activators of capsaicin-sensitive transient receptor potential vanilloid type 1 (TRPV1) receptor present on C-fibres, 0.9% acetic acid solution was used as an irritant (intraperitoneal injection, ip). The analgesic activity of LPP1, capsazepine (TRPV1 antagonist) and indomethacin was assessed in two groups of animals, i.e. mice with preserved and mice with destroyed C-fibres.
Capsazepine (40 mg/kg, subcutaneous injection, sc) was antinociceptive only in animals with C-fibres kept, whereas it insignificantly reduced nocifensive writhings in the second group of animals (42% vs. 8% in comparison with the control groups). Both LPP1 (30 mg/kg, ip) and indomethacin (10 mg/kg, sc) were potent analgesics in every group of mice, diminishing the number of writhes for approximately 87–89% and 94–98%, respectively, which proved their dual mechanism of antinociceptive action: C-fibres-dependent as well as C-fibres-independent.

On the basis of available data (anticonvulsant activity of LPP1 in maximal electroshock test and local anesthesia test) this C-fibres-independent pathway of its analgesic activity can be linked to the influence on voltage-gated sodium channels present on the surface of different types of nerves involved in the transmission of painful stimuli towards the central nervous system.

Effect of omeprazole on oral bioavailability and pharmacokinetics of vinpocetine in rats

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Background: Previous surveys proved that food strongly enhance bioavailability of vinpocetine. There are no studies evaluating the influence of PH alteration in gastrointestinal tract on vinpocetine pharmacokinetics during proton inhibitor pump treatment.

Objectives: The aim of this study was to evaluate the influence of omeprazole on the pharmacokinetics of vinpocetine.

Methods: This single-dose, parallel study involved male Wistar rats. Animals were divided into 2 groups. First group (V group) received single oral dose of vinpocetine (2 mg/kg b.w.) orally. In the second group (OV group) omeprazole was administered intraperitoneally at a dose of 10 mg/kg b.w. once daily from 1st to 5th day of experiment. On 6th day rats received single oral dose of vinpocetine (2 mg/kg b.w.). For analysis of vinpocetine pharmacokinetic properties, including Cmax, AUC0–t, and AUC0–∞, blood samples were obtained before and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 hours after administration. Vinpocetine concentrations were measured by high-performance liquid chromatography.

Results: The mean (SD) AUC0–t (ng h/ml), AUC0-inf (ng h/ml) and Cmax (ng/ml) in V group were 504.0 (57.3), 524.6 (56.7), and 135.3 (11.7), respectively. The corresponding values in OV group were 483.5 (42.4), 499.5 (43.6), and 149.0 (13.9), respectively. There were no statistically significant differences between V group and OV group (p > 0.05). Omeprazole did not affect the pharmacokinetic profile of vinpocetine indicating no influence of PH changes of gastrointestinal tract on bioavailability of vinpocetine.
Antiarrhythmic activity of novel pyrrolidin-2-one derivatives with adrenolytic properties

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Ventricular fibrillation (VF) is a major cause of death in acute myocardial infarction, in both the pre-hospital and in-hospital phases. The effective prevention of VF is therefore a most important aspect of the management of patients with acute myocardial infarction. At present has not completely effective and safe antiarrhythmic agents in these arrhythmias.

Our earlier research showed that some pyrrolidin-2-one derivatives had marked significant antiarrhythmic (adrenaline-induced) and hypotensive acts. This compounds had affinity for α₁- and α₂-adrenoceptors and antagonized the pressor response elicited by epinephrine, norepinephrine and methoxamine. The observed effect suggested that these compounds had adrenolytic properties [Malawska B et al., Eur J Med Chem, 2002; Malawska B et al., Il Farmaco, 2005; Kulig K et al., Arch Pharm Chem Life Sci, 2007].

As a continuation of this study, a series of novel pyrrolidin-2-one derivatives with adrenolytic properties was evaluated for antiarrhythmic, electrocardiographic and hypotensive activity. Some of them displayed antiarrhythmic activity in adrenaline and barium chloride induced arrhythmia and in the rat coronary artery ligation-reperfusion model. This compounds slightly decreased the heart rate, prolonged P-Q, Q-T intervals and QRS complex. Among them, compound EP-40 (1-[2-hydroxy-3-[4-[(2-hydroxyphenyl)-piperazin-1-yl]-propyl]-pyrrolidin-2-one showed excellent antiarrhythmic activity. This compound had significantly antioxidant effect, too. The present results suggest that the antiarrhythmic effect of compound EP-40 is related to their adrenolytic and antioxidant properties.

The role of the brain noradrenergic system in the regulation of cytochrome P450 expression in rat liver

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The aim of the present study was to ascertain whether the brain noradrenergic system may affect the expression of liver cytochrome P450 (CYP). In a screening study, rats were injected intraperitoneally with the noradrenergic neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4). One week after neurotoxin injection the levels of neurotransmitters (noradrenaline, dopamine, serotonin) and their metabolites were measured in different areas of the brain (the hypothalamus, cortex, striatum, hippocampus, cerebellum and the brain stem), and the activity and protein levels of CYP isoforms were measured in liver microsomes. In the brain, DSP-4 selectively decreased noradrenaline levels, not affecting dopamine or serotonin ones. The applied neurotoxin evoked decreases in the activity of CYP2B, CYP2C11 and CYP3A, while the activity of CYP2A, CYP2C6 and CYP2D was not affected. The observed changes in CYP activity did not always positively correlate with alterations in the expression of protein levels, which might be due to an additional peripheral effect of the neurotoxins. Studies on the local, intraventricular administration of noradrenergic neurotoxins to the brain (DSP-4, 6-hydroxydopamine) confirmed the role of the brain noradrenergic system in the regulation of cytochrome P450 expression in rat liver.
Action of quercetin-5'-sulfonic acid sodium salt (NaQSA) on ADMA/DDAH pathway in extracorporeal liver perfusion (ELP) in rats

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Background: N(G),N(G)-dimethyl-L-arginine (ADMA), an endogenous inhibitor of NO synthases, is metabolized by dimethylarginine dimethylaminohydrolase (DDAH) – an enzyme located mainly in liver. Influence of quercetin – a popular flavonoid – on NO production is not fully understood and may be due to its influence on ADMA metabolism.

Aim: The study was designed to investigate effect of water-soluble derivative of quercetin – quercetin-5'-sulfonic acid sodium salt (NaQSA) on ADMA/DDAH pathway during extracorporeal liver perfusion in rats.

Methods: The study was carried out on Wistar rats which were divided into 4 groups. Livers in group C were perfused with Krebs-Henseleit bicarbonate buffer (KHB) + ADMA (1 µM), in Q10 – with KHB + ADMA (1 µM) + NaQSA (10 µM), in Q50 – with KHB + ADMA (1 µM) + NaQSA (50 µM), and in group 0 (sham) – with KHB alone. Rats were anesthetized (thiopental, 70 mg/kg) and livers were excised and perfused in an open-circuit mode with 30 ml/min of flow rate. During perfusion fluid were collected to assay ADMA levels and aspartate and alanine aminotransferases (AST, ALT). After 90 min the perfusion livers were homogenized on ice and supernatants were taken to assay DDAH activity.

Results: In group Q50 AST activity was significantly lower than in group C at the end of perfusion (p < 0.05), and DDAH activity was lower than in group Q10 (p < 0.05). Significant difference between group Q10 and C was also observed (p < 0.05). Significant influence of duration of perfusion on ADMA levels was shown (p < 0.001). In 15 and 45 min. of perfusion ADMA value in group Q10 was higher than in C (p < 0.05 in both time points). During perfusion the greatest decrease in ADMA level was observed in group Q10 and the lowest in group Q50 with difference on the significance border (p = 0.06). In group 0 (sham) ADMA values were on the border of sensitivity of the analytical method and no significant differences were observed.

Conclusions: We have demonstrated that NaQSA at the concentration of 50 µM exhibited the strongest protective effect on liver function. But protective effect on DDAH/ADMA pathway was observed when NaQSA was used in lower (10 µM) concentration – higher DDAH activity compared to control group and the highest decrease of ADMA was noticed Impact of endogenous ADMA is negligible because its levels in sham group were very low and during the whole perfusion were on the same level.
Action of quercetin-5’-sulfonic acid sodium salt (NaQSA) on SOD activity and liver function in extracorporeal liver perfusion (ELP) in rats

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Background: Quercetin is a popular flavonoid with many beneficial properties such as antitumor, antiviral, antithrombotic and vasodilatory effect. It reduces liver injury in many diseases and exerts antioxidant activity, with mechanisms involving both free radical-scavenging and metal chelation.

Aim: The study was designed to investigate effect of water-soluble derivative of quercetin – quercetin-5’-sulfonic acid sodium salt (NaQSA) on liver function nod SOD activity during extracorporeal liver perfusion in rats.

Methods: The study was carried out on Wistar rats which were divided into 3 groups. Livers in group C were perfused with Krebs-Henseleit bicarbonate buffer (KHB) + ADMA (1 µM), in Q10 – with KHB + ADMA (1 µM) + NaQSA (10 µM), and in Q50 – with KHB + ADMA (1 µM) + NaQSA (50 µM). Rats were anesthetized (thiopental, 70 mg/kg) and livers were excised and perfused in an open-circuit mode with 30 ml/min of flow rate. During perfusion fluid were collected to assay aspartate and alanine aminotransferases (AST, ALT). After 90 min the perfusion livers were homogenized on ice and supernatants were taken to assay SOD activity.

Results: The study showed significant influence of duration of perfusion on the activities of ALT and AST (p < 0.001 for both comparisons). AST activities were the lowest in group Q50 in all time points of perfusion. In 45 min. they were significantly lower in Q10 and Q50 than in C (p < 0.05 in both cases). In 90 min. AST activity in Q50 was also significantly lower than in group C (p < 0.05).

In contrast to group C no increase in ALT and AST activities between 15 and 45 minute of perfusion was observed in group Q10 and Q50 (for AST p < 0.005 for both comparisons, and for ALT p < 0.05 for both comparisons). Increase in AST activity between 15 and 90 minute of the perfusion was significantly lower in group Q50 than in group C (p < 0.05). SOD activity was the highest in Q50 and was significantly different (p < 0.05) from the values obtained in group Q10.

Conclusion: We have demonstrated that NaQSA in the concentration of 50 µM exhibited the strongest protective effect on liver function what was indicated by liver enzymes activities and on oxido-redox status with the highest SOD activity.
The effects of acute and chronic administration of dehydroepiandrosteron on the pain threshold and arterial blood pressure in male and female SHR and WKY rats

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Dehydroepiandrosteron (DHEA) is a neurosteroid, which plays an important role in many neurobiological processes. It is well known that DHEA inhibits GABA and activates NMDA and sigma-1 receptors, which are critically involved in the control of pain mechanisms. Acute administration of DHEA to rats causes a rapid pro-nociceptive effect, whereas chronic treatment with this neurosteroid results in the pro-antinociceptive effect. It has been suggested that androgenic metabolites of DHEA might be involved in the modulation of antinociception.

The aim of this study was to investigate the effects of acute and chronic administrations of DHEA on the pain threshold and arterial blood pressure of male and female spontaneously hypertensive (SHR) and normotensive Wistar Kyoto (WKY) rats.

DHEA at a dose of 10 mg/kg was administrated per os every day at the same time. Arterial pressure was measured twice per week in each group of rats using a tail-cuff sphygmomanometer with an infrared sensor linked to a computer. Pain threshold was assessed daily by compression of the hind paw (mechanical method).

The most interesting finding of this study is, that acute (first) as well as chronic (once daily for 28 days) administrations of DHEA produced analgesic activity only in male SHR rats. Moreover, DHEA produced temporal hyperalgesia lasted throughout 2 first weeks of its daily administration to juvenile male WKY, but not SHR, rats. It seems that DHEA had no effect on the pain threshold in female SHR and WKY rats.

Results obtained in this study may suggest an involvement of endocrine system as well as mechanisms engaged in blood pressure control in the analgesic activity of DHEA. However, further investigation is needed.

The effects of *Phaseoli Pericarpium* extract on pro-atherosclerotic factors in rats with prediabetes

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Background: Accelerated atherosclerosis, the leading cause of death in modern societies, is related not only to overt diabetes, but is also significantly pronounced in prediabetic state. Therefore, any interventions in this early prediabetic stage which might slow the progression of atherosclerosis would be invaluable. *Phaseoli Pericarpium* extract (PPE) is expected to have beneficial effect on carbohydrates and lipid metabolism and protective effect against atherosclerosis so that the aim of the study was to investigate the effects of PPE on fatty acid profile and glucose tolerance in rats with prediabetes.
**Materials and Methods:** Male Wistar rats were divided into four groups: control (C): prediabetes (P): control (PC), metformin (PM), and phaseoli pericarpium extract (PPP). Prediabetes model was established by 3-week high-fat diet. Rats of control and prediabetes groups received water, metformin, or PPE daily by oral route for 4 weeks. Oral glucose tolerance test (OGTT) was performed twice, before and after completion of experiment. In each rat lipid and fatty acid profiles in abdominal adipose tissue were analysed.

**Results:** Treatment of prediabetic rats with PPE led not only to beneficial and comparable to metformin changes in fatty acid profile in abdominal adipose tissue (more polyunsaturated, n-3 and n-6 fatty acids), but also improved carbohydrates metabolism (glucose tolerance in OGTT).

**Conclusions:** These results suggest PPE as a useful instrument in the prophylaxis and therapy of atherosclerosis in prediabetic stage and it might partly attribute to reverse of glucose intolerance and pro-atherogenic fatty acid profile.

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**Cardio-respiratory effects of dermorphin-substance P chimeric peptide in anaesthetized rats: role of the lung vagi**

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It is generally accepted that systemic administration of opioids, besides analgesia, cause a variety of side effects like depression of ventilation, arterial hypotension and drug dependence, which narrow their clinical use.

Neuropeptide – substance P (SP) presents an excitatory transmitter in the control of respiratory drive and is likewise involved in modulation of pain.

The aim of this study was to determine the respiratory pattern produced by recently synthesized chimeric peptide termed AWL3106, comprising two pharmacophores with agonistic affinity to µ opioid (dermorphin) and tachykinin NK1 (SP) receptors.

We measured the cardiorespiratory parameters in 18 anaesthetized rats, breathing spontaneously room air, after an intravenous injection of 0.3 umol/kg AWL3106 while neurally intact and initially midcervically vagotomized.

Bolus injection of AWL3106 in neurally intact rats resulted in (i) respiratory depression comprising the apnoea of mean duration of 5 s, followed by breathing of 19% decreased rate (f), compensated by (ii) 22% augmentation in tidal volume (VT); (iii) lowered mean arterial pressure (MAP) by 61%.

Midcervical vagotomy prevented bradypnoeic response to AWL3106, halved the increase in VT and reduced by 26% the maximal fall in MAP. Moreover rats subjected to vagal denervation displayed a transient drop in tidal volume at 5 to 10 s after injection of the drug.

This study has shown that activation of the neuropeptide receptors with 3106 depressed only timing component of the respiratory pattern. Minute ventilation was unchanged due to compensatory augmentation in tidal volume. Lung vagal afferentation seems to be a crucial pathway for these effects.

Short-lived depression of tidal volume in denervated rats and post-vagotomy arterial hypotension may relate to the excitation of peripheral or central neuropeptide receptors occurring outside of the lung vagi.

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The contribution of human cytochrome P450 (CYP) isoenzymes to the metabolism of phenothiazine neuroleptics

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Our earlier studies indicated that differences in the structure of phenothiazine neuroleptics (mainly the structure of a side-chain) influenced their interaction with a catalytic site of CYP. CYP1A2 and CYP3A4 are the main isoenzymes responsible for the 5-sulfoxidation of promazine, perazine and thioridazine. However, some inter-drug differences were observed in the catalysis of the side-chain mono-N-demethylation: promazine – CYP1A2 and CYP2C19, perazine – CYP2C19 and thioridazine – CYP1A2 and CYP3A4 [Wójcikowski et al., Br J Pharmacol, 2003; Eur Neuropsychopharmacol, 2004; Drug Metab Dispos, 2006].

The aim of the present study was to identify CYP isoenzymes involved in the 5-sulfoxidation, mono-N-demethylation and di-N-demethylation of the aliphatic-type phenothiazine neuroleptic chlorpromazine in human liver. The experiments were performed in vitro using cDNA-expressed human CYP isoforms (Supersomes 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4), liver microsomes from different donors and CYP-selective inhibitors. The obtained results indicate that CYP1A2 is the only CYP isoform that catalyzes the mono-N-demethylation and di-N-demethylation of chlorpromazine (100%) and the main isoform responsible for chlorpromazine 5-sulfoxidation (64%) at a therapeutic concentration of the drug (10 mM). CYP3A4 contributes to a lesser degree to chlorpromazine 5-sulfoxidation (34%). The role of CYP2B6, CYP2C19 and CYP2D6 in the catalysis of the latter reaction is negligible (0.1–2%). In conclusion, the catalysis of chlorpromazine N-demethylation and 5-sulfoxidation in humans exhibits a stricter CYP1A2 preference compared to the previously tested phenothiazines. Hence pharmacokinetic interactions involving chlorpromazine and CYP1A2 substrates or inhibitors are likely to occur.

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Modeling dynamics of paroxysmal activity and pharmacokinetics of GABA_A receptor ligands during their direct application into their biophase of action

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Dynamics of paroxysmal activity development was studied during application of several GABA_A receptor modulators (pentylentetrazole, picrotoxin, bicuculline, strychnine) in different doses to the rat cerebral cortex. For the estimation of the power of cortical epileptic complexes that reflect epileptic activity of the brain, a new method of calculating this parameter has been proposed. The method is based on the use of THE parameter $W_i$ equal to the total deviation of the potential $U_i$ from the integral mean value ($U_{av}$) over a fixed time period:

$$U_{av} = \frac{1}{N} \sum_{i=1}^{N} U_i, \quad W_i = \sum_{i=1}^{N} |U_i - U_{av}|$$
where: $W_i$ is the brain electrical activity power, $U_{av}$ and $U_i$ – average and $i$ – potentials respectively, $N$ – number of recorded spikes.

We developed mathematical models that enabled determining pharmacokinetic parameters of drugs from their pharmacodynamic data during direct administration into the biophase of their action. We have demonstrated that pharmacokinetic scheme of these convulsant compounds conformed to the catenary multi-compartment model. The parameters of this model are determined by two irreversible first-order processes, absorption and elimination. Hypothesis about irreversibility of the kinetic scheme of the drug mass transfer in conditions of its direct application into the biophase of action was experimentally confirmed.

Comparative analysis of pharmacokinetic data of these drugs was carried out. Pharmacological effects of picrotoxin and bicuculline appeared to be transient, i.e. strictly determined by their concentrations in the brain. Cooperative effect was noted for pentylenetrazole and strychnine. The proposed approach to modeling the pharmacokinetic-pharmacodynamic relationship enhances capabilities of this experimental model in electrophysiological and biopharmaceutical studies.