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Session I
Antioxidants in lifestyle-related diseases

MECHANISMS OF ANTIOXIDANT THERAPY IN HYPERTENSION: THE HYPOTHESIS

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Endothelial dysfunction plays a substantial role in the pathogenesis of hypertension. The imbalance between reactive oxygen species (ROS) and nitric oxide (NO) generation has been proposed to be a decisive cause of endothelial dysfunction and vascular injury. However, despite the fact that elevated reactive oxygen species production was documented in different forms of hypertension, clinical studies on chronic antioxidant therapy of hypertension fail to bring any beneficial effect. This discrepancy may be partially determined by different effects of short- and long-lasting treatment with antioxidants or scavengers. Elevated ROS production in hypertension need not be only harmful. It also stimulates the activity of the antioxidant defense system and improves the NO/cGMP pathway, resulting in establishment of a new equilibrium between enhanced oxidative load and stimulated NO-pathway, thus maintaining sufficient NO bioavailability. It has been suggested that antioxidant treatment might be beneficial for a short time, until increased NO generation predominates over ROS production. Further weakening of ROS formation by antioxidants may attenuate nuclear factor-κB activation resulting in decreased endothelial NO synthase expression and activity. Thus, prolonged antioxidant therapy may attenuate the beneficial regulatory effect of ROS leading to decreased NO generation and reestablishment of the undesirable disproportion between deleterious and protective forces. As a consequence prolonged antioxidant treatment in human hypertension may fail to provide long-lasting beneficial effect.

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THE EFFECT OF FLAVONOIDS ON THE LEVEL OF Cu/Zn SOD AND NOS PROTEINS IN EXPERIMENTAL DIABETES

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Both experimental and clinical studies suggest that oxidative stress plays an important role in the pathogenesis of diabetes mellitus. Hyperglycemia leads to free radical generation and modulation of nitric oxide (NO) level as well as the activity of antioxidant enzymes, like Cu/Zn superoxide dismutase (SOD) what could contribute to the deleterious consequences of diabetes mellitus and cause neural degeneration [1,2]. NO contributes to the regulation of cerebral circulation and participates in the regulation of brain functions. The interaction between NO and superoxide results in formation of peroxynitrite, a strong oxidant. This reaction represents one of the important mechanisms implicated in the neurological complications of diabetes, because it reduces bioavailability of NO. The aim of this study was to investigate the possible neuroprotective effects of flavonoids from *Pinus Pinaster* realized through determination of protein level (neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS) as well as Cu/Zn SOD) in cerebral cortex after 7 weeks of daily administration of flavonoids (10 mg/kg/day). To determine the amount of immunodetectable nNOS, eNOS and Cu/Zn SOD proteins we used Western blot analysis. According to our results level of proteins nNOS and Cu/Zn SOD are decreased in cerebral cortex of diabetic rats in comparison to control group and administration of flavonoids significantly increased this level. The effect on eNOS was not observed. Found alterations of Cu/Zn SOD and nNOS may be one of the important factors in the vulnerability of the brain to free oxygen radicals and may be related to the pathophysiology of neurological complications. This study demonstrates that flavonoids could protect the neuronal tissue against the oxidative damage through increasing the synthesis of Cu/Zn SOD and nNOS proteins.

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THE PREVENTIVE EFFECT OF MELATONIN ON LEFT VENTRICULAR REMODELING INDUCED BY L-NAME TREATMENT IN RATS: DISSOCIATED EFFECT ON FIBROSIS AND HYPERTROPHY

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Due to the hypotensive, antioxidative and NO enhancing properties of melatonin, it was supposed that it might have antiremodeling effects. We have investigated, whether melatonin was able to prevent LV hypertrophy and fibrosis associated with L-NAME (NG-nitro-L-arginine-methyl ester)-induced hypertension. 4 groups (n=10 in each) of male adult Wistar rats were investigated: control, L-NAME (50 mg/kg), melatonin (10 mg/kg) and L-NAME + melatonin. Blood pressure was measured non-invasively each week. After 5-week treatment, the animals were sacrificed and the NO-synthase (NOS) activity, endothelial and inducible NOS expression, level of collagenous proteins, hydroxyproline and conjugated dienes (CD) in the LV were determined. L-NAME administration inhibited NOS activity, increased CD concentration, elevated blood pressure and induced LV hypertrophy and fibrosis. Addition of melatonin to L-NAME treatment failed to prevent the attenuation of NOS activity and the development of LV hypertrophy and prevented hypertension only partially. Yet, melatonin administration completely prevented the increase in CD concentration and the development of LV fibrosis in rats treated with L-NAME. We conclude that the selective prevention of LV fibrosis but not hypertrophy in L-NAME hypertensive rats might be linked to its antioxidant properties and independent of NOS activity.

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THE EFFECT OF PYCNOGENOL ON LEVEL OF NO AND NITROTYROSINE IN PATIENTS WITH DIABETES MELLITUS SUFFERING FROM ERECTILE DYSFUNCION

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Erectile dysfunction (ED) is highly prevalent in diabetes mellitus. Pathophysiological mechanisms of diabetes-associated ED are related to

endothelial dysfunction. Endotelial nitric oxide (NO) plays essential role in the early steps of relaxation of the penile vasculature and cavernous smooth muscle cells. Additionally, increased auto-oxidation of glucose and oxidation of low-density lipoproteins known in diabetes resulting in the overproduction of free radical species, may lead to smooth muscle dysfunction [1]. We evaluated the possibility of ED treatment in diabetic patients by Pycnogenol (Pyc), the extract from pine bark of *Pinus pinaster*. Pyc is very powerful antioxidant and stimulates endothelial NO-synthase for enhanced production of NO. Randomized, double-blind and placebo controlled pilot study included 15 diabetic men with ED. Patients were treated by Pycnogenol (120 mg/day) or Placebo for three months. The erectile function was investigated by IIEF – 5 questionnaire (International Index of Erectile Function), total antioxidant status (TAS) (TEAC method), malone-dialdehyde (MDA) by HPLC, total NO as nitrites with set (R & D System, Germany) and nitrotyrosine (NT) (ELISA,Hbt., Netherlands). Results: Pyc caused a significant improvement of sexual functions in diabetic men with ED (p(Pyc/Pl)=0,025, p(0/3)=0,029). MDA level was marginally decreased (p(Pyc/Pl)=0,095, p(0/3)=0,08) whereas TAS, NT and NO were not significantly changed after three months of Pyc administration. Our data allow us to conclude, that Pycnogenol improves the erectile function in DM patients by its ability to decrease oxidative damage to lipids and to protect endotelium from oxidative stress.

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PROVINOL AND STRESS-INDUCED HYPERTENSION IN THE BORDERLINE HYPERTENSIVE RATS

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Provinol (red wine polyphenolic compounds) has been reported to reduce the augmented contractions of the aorta to noradrenaline in rats with NO-deficient hypertension (1). The aim of this study was to investigate the effect of long-term administration of provinol on blood pressure and contractile responses of the isolated superior mesenteric artery to adrenergic stimuli in borderline hypertensive rats (BHR) exposed to 8-week lasting crowding stress. Adult, male normotensive Wistar-Kyoto (WKY) rats and BHR (F1 offspring of spontaneously hypertensive dams and WKY sires) were divided into four groups: control (480 cm^2/rat), provinol-treated (20 mg/kg/day, 480 cm^2/rat), stressed (200 cm^2/rat) and stressed treated with provinol (20 mg/kg/day, 200 cm^2/rat) for 8 weeks. Systolic blood pressure (BP) was measured by the tail cuff plethysmography. Rings of the isolated superior mesenteric artery were mounted in organ baths for measurements of isometric contractile force. Neurogenic responses of mesenteric artery were elicited by electrical stimulation of perivascular nerves. Resting blood pressure of BHR was 140.0±2.3 mm Hg, which was higher than that of Wistar rats (110.7±2.3 mm Hg, $P<0,05$). Chronic crowding stress increased values of BP in BHR to 149.1±1.1 mm Hg. Simultaneous administration of provinol to stressed BHR prevented elevation of BP. Provinol alone had no significant effect on adrenergic responses of mesenteric artery induced by endo- and exogenous noradrenaline neither in unstressed BHR nor in unstressed Wistar-Kyoto rats. However, provinol in combination with stress produced significant enhancement of adrenergic responses as compared to stressed alone. Contractile responses of mesenteric artery to exogenous noradrenaline were slightly reduced in stressed BHR compared to their unstressed controls. Simultaneous administration of provinol to stressed BHR prevented the reduction of noradrenaline-induced contractions. The improvement of stress-induced reduction of noradrenaline contractions in BHR by provinol seems to be the consequences of so far unspecified properties of polyphenols.

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PHARMACOLOGICAL PROTECTION OF MYOCARDIAL REMODELATION IN THE SPONTANEOUSLY HYPERTENSIVE RATS

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Left ventricular hypertrophy is an adaptive response to excessive hemodynamic overload. However, it represents an independent risk factor of increased cardiovascular morbidity and mortality. Melatonin was suggested to have antiremodeling properties, due to its antioxidative and scavenging effects, endothelium and nitric oxide (NO) preserving action and hypotensive effect. We have compared the effect of melatonin, captopril and spironolactone on the regression of left ventricular (LV) remodeling and hypertension in spontaneously hypertensive rats (SHR). Five groups (n=10 in each) of male adult Wistar and SHR rats were investigated: control Wistar rats, SHR, SHR+captopril (100 mg/kg/24h), SHR+spironolactone (200 mg/kg/24h) and SHR+melatonin (10 mg/kg/24h). Blood pressure was measured non-invasively each week. After 5-week treatment, the animals were sacrificed and the relative heart weight, level of collagenous proteins, NO-synthase (NOS) activity and nuclear factor κB (NF-κB) expression in the left ventricle were determined. We observed, that SHR showed hypertension, LV hypertrophy and fibrosis associated with increased NOS activity and NF-κB expression in LV. Systolic blood pressure was reduced by captopril (most effectively), spironolactone and melatonin administration. All drugs slightly increased NOS activity in LV. Melatonin administration reduced NF-κB expression in LV and both captopril and melatonin treatment affected LV remodeling. While captopril reduced LV hypertrophy, only melatonin decreased the level of collagenous proteins in LV. We conclude that LV weight reduction is associated mainly with intensive blood pressure decrease (attained by captopril treatment), while melatonin induced fibrosis decrease coincides with its massive antioxidant effect. Although melatonin administration has not reverted LV hypertrophy, the hypertrophied myocardium with reduced level of fibrosis might be accompanied by neither increased rigidity and dysfunction of LV, nor electrical instability. Therefore a physiological regression of LV hypertrophy might be achieved.

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Session II
Mechanisms of regulation of NO synthesis and release

TIME- AND DOSE-DEPENDENT MODULATION OF NITRIC OXIDE PRODUCTION WITH L-NAME IN BORDERLINE HYPERTENSIVE RATS

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Non-specific nitric oxide synthase (NOS) inhibitor N^G-Nitro-L-arginine methyl ester (L-NAME) reduces nitric oxide (NO) production, endothelium-dependent vasorelaxation and induces hypertension in normotensive rats when it is administered in relatively high dose (more than 5 mg/kg/day). However, administration of a low dose of L-NAME (1.5 mg/kg/day) for 8 weeks activated NO production in the cardiovascular system of Wistar rats, while no effect was seen in corresponding borderline hypertensive rats. These findings led to the hypothesis that NO production can be modulated *in vivo* by appropriate dose of L-NAME and time of treatment. The aim of this study was determine alterations in blood pressure (BP) and NO production in borderline hypertensive rats (BHR) after chronic low-dose L-NAME-treatment. Adult male, 12-week-old, BHR were treated with L-NAME (1 and 0.3 mg/kg/day, respectively) in drinking water for 4 or 10 weeks. BP was determined non-invasively by tail-cuff method. NOS activity was determined by conversion of [^{3}H]-L-arginine to [^{3}H]-L-citrulline in

the aorta, left ventricle (LV), kidney, liver, hypothalamus and cerebellum. L-NAME at the dose of 1 mg/kg/day significantly elevated BP already after two weeks of treatment. Lower dose of L-NAME (0.3 mg/kg/day) failed to affect BP significantly. Body weight and relative left ventricular mass were unchanged by L-NAME-treatment. Four weeks of L-NAME 1 mg/kg/day significantly reduced NOS activity in the aorta, liver, hypothalamus and cerebellum and partial reduction was seen in the LV and kidney (p=0.08 vs. age-matched control). The extension of this treatment for 10 weeks resulted in significant activation NOS vs. controls in the aorta, LV, liver and kidney. Significant reduction was still seen in the hypothalamus. Lower dose of L-NAME (0.3 mg/kg/day, 10 weeks) activated NO production only in the LV and kidney (vs. control), had no effect in the aorta and liver, and reduced NO production in both the hypothalamus and cerebellum. In conclusion, results suggest that NO production can be increased in the given peripheral tissues by 10 weeks-lasting administration of L-NAME in the dose of 1 mg/kg/day also in pre-hypertensive conditions. However, this mechanism failed to reduce BP or relative left ventricular mass in rats.

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EVIDENCE FOR ALTERED FEEDBACK REGULATION OF NITRIC OXIDE SYNTHESIS IN HYPERTENSIVE RATS

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Chronic pharmacological reduction of nitric oxide synthesis with the non specific NO synthase (NOS) inhibitor N^G-Nitro-L-arginine methyl ester (L-NAME) results in reduction of endothelium-dependent vasorelaxation and hypertension when it is administered in higher dose (more than 5 mg/kg/day). We provided evidence for the negative-feedback regulatory role of NO on NOS activity in Wistar rats by administration of a low dose of L-NAME (1.5 mg/kg/day), but there is no information about this regulatory mechanism in hypertensive rats (1). Thus, the aim of this study was examine the modulation of vascular NO production and NO-dependent and independent components of endothelial function by chronic low-dose L-NAME treatment in Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats. Adult male 12-week-old WKY and SHR rats were treated with L-NAME (0.3 mg/kg/day) in drinking water for 10 weeks. Blood pressure (BP) was determined by tail-cuff method. NOS activity was determined by conversion of [^{3}H]-L-Arg to [^{3}H]-L-Cit in the aorta. NO-dependent component of endothelium-dependent relaxation was investigated in the femoral artery using the wire myograph during isometric conditions, as a difference between acetylcholine (ACh)-induced relaxation before and after acute L-NAME (300 µmol/l) pre-treatment. Since vasorelaxation, expressed as a percentage of pre-constriction, was inversely related to initial tension, relaxing responses were expressed as absolute values (mN/mm) too. Chronic low-dose of L-NAME failed to affect BP and relative as well as absolute ACh-induced vasorelaxation in WKY. In SHR, chronic L-NAME significantly elevated BP, but failed to affect ACh-induced vasorelaxation. However, in WKY chronic L-NAME administration increased both relative and absolute NO-dependent component and decreased L-NAME-resistant component of ACh-induced relaxation, which was not observed in SHR. Similarly, low-dose of L-NAME had no effect on NOS activity in the aorta of SHR, but it increased NO production in WKY. In conclusion, chronic low-dose L-NAME treatment increased NO production in normotensive rats, supposedly by negative feedback regulation, but this regulatory mechanism was failed in hypertensive rats.

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H_2S RELEASED NO FROM S-NITROSOGLUTATHIONE, EFFECTS OF LIPIDS

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Endogenously produced H_2S is involved in neuromodulation, cell proliferation, apoptosis, regulation of cardiac function and cardioprotection, vasorelaxation, hypertension, and septic, endotoxin and haemorrhagic shocks and inflammation processes [1,2]. Molecular mechanisms of the numerous H_2S effects are not fully understood. Recently we observed that H_2S/HS^- releases NO from S-nitrosoglutathione [3]. However, a molecular mechanism and an effect of biological compounds on the release is not known. Therefore we studied the effects of H_2S donor NaHS on blood pressure and heart rate of anesthetized rats. NaHS (up to 64 µmol/kg, i.v.), a donor of H_2S and HS^-, decreased transiently the blood pressure to (84.5-46.5) %, followed by its increase to (107.8-170.8) %. We characterized the effect of H_2S on single channel properties of chloride channels derived from rat heart lysosomal vesicles and RyR2 calcium channels derived from rat heart sarcoplasmic reticulum incorporated into a bilayer lipid membrane. H_2S inhibited the chloride channels, but transiently activated RyR2 channels, which may explain its effect on the blood pressure. Since H_2S/HS^- releases NO from S-nitrosoglutathione, we studied effect of membrane lipids on the NO release. We found that lipids having unsaturated fatty acids, isolectin, 1,2-dioleoyl-sn-glycero-3-phosphocholine, and 1,2-dioleoyl-sn-glycero-3-phosphoserine depressed the H_2S/HS^- induced NO release from S-nitrosoglutathione. On the other hand, lipid having saturated fatty acids, dipalmitoyl phosphatidolcholine, did not influence the NO release. We assume that the saturation of lipid fatty acyl chains and lipid composition of biological membranes modulates NO release from nitrosoglutathione induced by H_2S/HS^-.

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EFFECT OF NO RELEASING AGENT H_2S ON CHLORIDE AND RYR2 CALCIUM RELEASE CHANNEL

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Hydrogen sulfide, H_2S, is an endogenously formed gaseous transmitter. H_2S is involved in a regulation of cardiovascular functions in an ischemia/reperfusion preconditioning and postconditioning or hypertension. It affects L-type Ca^{2+} and K_{ATP} cardiac ion channels. However its effect on other channels is unknown. Since we found that H_2S induced release of NO from nitroso-proteins, we decided to study the effect of H_2S on the activity of chloride and ryanodine receptor Ca^{2+} release channels (RyR2) derived from lysosomal vesicles and cardiac sarcoplasmic reticulum, respectively. The chloride channels derived from the rat heart lysosomal vesicles were incorporated into a bilayer lipid membrane (BLM). The single chloride channel currents were measured in 250:50 mmol/l KCl *cis*/*trans* solutions. H_2S donor NaHS inhibited the chloride channels by decreasing the channel open probability (P-open) in a concentration-dependent manner. The inhibitory effect of H_2S was side-dependent with the IC_{50} values of 42 and 75 µmol/l for the *trans* and the *cis* sides, respectively. Single channel properties of the RyR2 channels, incorporated into BLM, were also modulated by H_2S. H_2S had a biphasic effect on P-open of the channel. H_2S donor NaHS increased 3.6-fold open probability of the RyR2 channel at 50 µmol/l. At higher concentrations (500-1000 µmol/l), in 6/11 experiments NaHS decreased P-open, while in 5/11 experiments, it did not have effect. We assume that the inhibitory effect of H_2S on chloride channels and its biphasic effect on RyR2 channels may be responsible for some of its numerous biological effects and that NO released from the nitroso-proteins may be also responsible for the effects.

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Session III
Nuclear factor kappa B in regulation of NO and blood pressure

THE ROLE OF NUCLEAR FACTOR κB IN THE PRODUCTION OF NITRIC OXIDE AND BLOOD PRESSURE REGULATION IN L-NAME-INDUCED HYPERTENSION

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The aim of our study was to elucidate the effect of NF-κB inhibition by lactacystine in L-NAME-induced hypertension. Adult 12-week-old male Wistar rats were subjected to treatment with L-NAME (40 mg/kg/day) for seven weeks. Half of rats received lactacystine together with L-NAME for last three weeks. Next 16-week-old male Wistar rats received lactacystine only for 3 weeks. Blood pressure (BP) was measured by tail-cuff plethysmography. Total NOS activity was determined by measuring the formation of L-[^{3}H] citrulline from L-[^{3}H] arginine, endothelial NOS and NF-κB (p65) protein by Western blot analysis and concentration of conjugated dienes (CD) spectrophotometrically. 7-week-L-NAME treatment increased BP comparing the age-matched untreated animals, while lactacystine treatment decreased it. Addition of lactacystine to the L-NAME increased BP significantly comparing both the untreated control and L-NAME groups. NOS activity in the L-NAME+lactacystine group was decreased in the left ventricle and there was decreasing tendency in the aorta. NOS activity already inhibited by L-NAME was not further changed by lactacystine treatment. There were no changes either in eNOS or NF-κB (p65) protein expression in L-NAME+lactacystine respective group. Lactacystine potentiated the effect of L-NAME in increased CD concentration, however, it faild to affect CD concentration in the brain. In conclusion, our results did not confirm a definite role of NF-κB in nitric oxide production in L-NAME-induced hypertension, however, it implied a regulatory effect which seems to be tissue-dependent. Further increase of blood pressure in L-NAME-induced hypertension after lactacystine treatment outline an important regulatory role of NF-κB in the maintenance of blood pressure in L-NAME-induced hypertension.

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THE EFFECT OF NUCLEAR FACTOR KAPPA B INHIBITION ON VASORELAXATION IN L-NAME-INDUCED HYPERTENSION

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Recently we have demonstrated involvement of NF-κB in the upregulation of endothelial nitric oxide synthase (eNOS) in hypertension induced by N^G-nitro-L-arginine methyl ester (L-NAME). Thus, the goal of our study was to analyze an effect of NF-κB inhibitor, lactacystine, on relaxation responses of femoral artery in L-NAME-induced hypertension. Adult 12-week-old male Wistar rats were treated with L-NAME (40 mg/kg/day) for seven weeks (n=14). Half of the rats received lactacystine together with L-NAME for last three weeks. Next 16-week-old male Wistar rats received lactacystine only for 3 weeks (n=7). Blood pressure was measured by tail-cuff plethysmography. Acetylcholine-induced relaxation responses of femoral artery were analyzed by Mulvany-Halpern myograph and NOS activity was determined by measuring the formation of L-[^{3}H] citrulline from L-[^{3}H] arginine. Seven-week L-NAME-treatment increased blood pressure by 38 % in comparison to age-matched untreated animals. Lactacystine alone treatment decreased blood pressure by 10 %. On the other hand, addition of lactacystin to the L-NAME treatment led to further increase of blood pressure (by 54 % compared to the untreated control group and by 12 % compared to the L-NAME group). Interestingly, 7-week-L-NAME treatment did not decrease NOS activity which corresponded well with unchanged relaxation responses of femoral artery in this group. Acute addition of L-NAME ($3x10^{-4}$ mol/l) to the incubation medium increased maximal relaxation of arteries isolated from the L-NAME treated group, while the relaxation responses in the L-NAME+lactacystine treated group remained unchanged. We hypothesized that the NF-κB inhibitor, lactacystine, may block upregulation of eNOS protein expression/activity activated as the compensatory mechanism due to the long-term L-NAME treatment. Further increased of blood pressure and decreased maximal artery relaxation after addition of lactacystine to the L-NAME treatment corresponds well with this hypothesis.

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INHIBITION OF NUCLEAR FACTOR KAPPA B MODIFIES NITRIC OXIDE SYNTHASE ACTIVITY IN BORDERLINE HYPERTENSION

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Since involvement of nuclear factor kappa B (NF-κB) in the upregulation of endothelial nitric oxide synthase (eNOS) in hypertension induced by N^G-nitro-L-arginine methyl ester was demonstrated, the goal of our study was to analyze an effect of NF-κB inhibitor, JSH-23 (4-methyl-N^1-(3-phenylpropyl)benzene-1,2-diamine), on blood pressure and NOS activity in borderline hypertensive rats (BHR). Rats were divided into four groups: 6- and 12-weeks old BHR treated with JSH-23 (10 μM, i.v.) for next 2 weeks and age-matched controls. Blood pressure was measured by tail-cuff plethysmography every week. NOS activity was determined by measuring the formation of L-[^{3}H] citrulline from L-[^{3}H] arginine in the aorta, heart, kidney and brain. At the end of treatment blood pressure was decreased by 24 % in young and 11 % in adult BHR treated with JSH-23 in comparison to age-matched untreated rats. NOS activity in the young control BHR was significantly higher in comparison to the adult control BHR in all tissue investigated. JSH-23 treatment decreased NOS activity of young BHR while it increased the activity in adult BHR in all tissues investigated. We hypothesized that in young BHR, NF-κB activation leads to increased NOS expression followed by increased activity which may represent a compensatory mechanism activated due to the blood pressure increase. After blood pressure stabilization NF-κB activation was decreased following by decreasing NOS activity. In the condition of sufficient NO production, inhibition of NF-κB led to decreased NOS activity, while in NO deficiency it elicited the opposite effect. Since NF-κB inhibition led to decreased blood pressure in both young and adult BHR, the involvement of NF-κB in blood regulation should include more than NO-mediated mechanism.

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Session IV
Vascular function in hypertension I

THE ROLE OF CYCLIC NUCLEOTIDES IN VASODILATION IN SPONTANEOUSLY HYPERTENSIVE (SHR) AND NORMOTENSIVE WISTAR RATS

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High blood pressure (BP) of SHR is attributed to enhanced activity of sympathetic nervous system (SNS) and/or impaired vasodilator action of nitric oxide (NO). Since norepinephrine (NE) dose-response curves do not differ in conscious SHR and Wistar rats, a high dependence of BP in SHR on SNS could result from excessive catecholamine secretion and/or the dysbalance between alpha-adrenergic vasoconstriction and beta-adrenergic vasodilation. A significant reduction of sympathetic vasoconstriction and decreased Ca^{2+} entry through voltage-dependent Ca^{2+} channels was found after chronic inactivation of inhibitory G

proteins by pertussis toxin leading to enhanced cAMP production, the effects being greater in SHR. This was accompanied by attenuated BP response to acute NO synthase blockade. We therefore investigated if BP response to acute L-NAME injection can be attenuated by cAMP overproduction. Beta-adrenoceptor stimulation was induced by isoprenaline infusion (100 ng/kg/min i.v.) which was followed by inhibition of NO formation with L-NAME (30 mg/kg i.v.) and subsequent injection of beta-adrenoceptor antagonist (propranolol, 1 mg/kg i.v.). The effect of NO donor sodium nitroprusside (20 μg/kg i.v.) was studied during acute NO deficiency. Isoprenaline infusion decreased BP in both rat strains (more in SHR) and prevented BP rise induced by acute inhibition of NO formation. The latter isoprenaline effect was completely reversed by propranolol so that fully developed BP response to L-NAME administration was observed. In contrast to the greater BP effects of increased cAMP levels in SHR, elevated cGMP levels following nitroprusside injection caused similar BP fall in both strains. In conclusions, enhanced cAMP production induced by either pertussis toxin injection or isoprenaline infusion causes greater BP reduction in SHR than in Wistar rats. This is associated with the attenuation of BP responses to acute NO deficiency indicating that overproduction of cAMP can compensate the lack of NO-dependent vasodilation and vice versa.

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VASORELAXING EFFECTS OF ACUTE CYCLOOXYGENASE INHIBITION: THE ROLE OF CALCIUM-ACTIVATED POTASSIUM AND CHLORIDE CHANNELS

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Norepinephrine (NE)-induced arterial contraction, which is augmented in the absence of NO, can be largely attenuated or prevented by cyclooxygenase (COX) inhibitors such as indomethacin. The aim of our study was to investigate the role of Ca^{2+}-activated K^+ or Cl^- channels in the vasorelaxing action of COX inhibitors. Isolated femoral arteries of Wistar rats (aged 6 months) were stimulated by NE (1-10 μM) in the absence or presence of L-NNA (100 μM). Indomethacin (10 μM) was given prior to or after the development of NE-induced contraction. Niflumic acid (10 μM) and tetraethylammonium (TEA, 1 mM) were used to evaluate the contribution of Ca^{2+}-activated Cl^- or K^+ channels. Both indomethacin and niflumic acid considerably attenuated arterial contraction elicited by NE in either NO absence or blockade of Ca^{2+}-activated K^+ channels. This attenuation was almost completely abolished by simultaneous presence of L-NNA and TEA in the incubation medium. In conclusions, our results suggest that vasorelaxing effects of COX inhibitors are highly dependent on the activity of Ca^{2+}-activated K^+ channels, which is stimulated by high intracellular calcium and/or by NO or EDHF action. The role of possible inhibition of Ca^{2+}-activated Cl^- channels in the attenuation of NE-induced contraction by COX inhibitors remains to be demonstrated by the use of other inhibitors of these channels. It also remains to determine whether these findings bear any relationship to the mechanisms of action of COX-dependent endothelium-derived contracting factor (EDCF).

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ALPHA-1- AND ALPHA-2-ADRENERGIC VASOCONSTRICTION IN WKY AND SHR RATS: THE EFFECT OF NIFEDIPINE

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Pressor effects of norepinephrine (NE) were reported to be enhanced in spontaneously hypertensive rats (SHR) compared to Wistar-Kyoto (WKY) rats, but NE dose-response curves recorded in conscious rats after the blockade of renin-angiotensin system (RAS) and sympathetic nervous system (SNS) are almost identical in both genotypes. Nifedipine shifted NE dose-response curves to the right in these two rat strains. The aim of our study was to determine the contribution of alpha-1- and alpha-2-adrenergic vasoconstriction to pressor effects of exogenous NE and the effect of nifedipine (0.4 mg/kg) on both adrenergic components in SHR and WKY. Blood pressure (BP) response to non-cumulative NE doses (0.01-20 μg/kg) was determined in conscious rats (pretreated with captopril and pentolinium) in the presence of alpha-1- or alpha-2-adrenoceptor antagonists (prazosin or yohimbine, 1 mg/kg each) before or after nifedipine injection. Dose-response curves were constructed from maximal BP increases reached after each NE dose. Yohimbine did not affect maximal BP response to NE in either genotype, but shifted NE dose-response curves to the right (more in SHR). In contrast, prazosin lowered maximal BP response more in WKY than SHR. Both antagonists decreased NE sensitivity in SHR only. In the presence of either antagonist nifedipine shifted NE dose-response curves to the right. Maximal pressor response to NE was lowered by nifedipine only when prazosin was present, the effect being more pronounced in SHR. In conclusions, the contribution of alpha-2-adrenergic mechanisms to NE pressor response is enhanced in SHR, while alpha-1-adrenergic contribution was similar in both genotypes. In SHR alpha-2-adrenergic component of NE-induced vasoconstriction was more suppressed by nifedipine than in WKY.

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Session V
Vascular function in hypertension II

THE ROLE OF OXIDATIVE STRESS PROTECTION IN ACETYLCHOLINE INDUCED RELAXATION OF ENDOTHELIUM-DEPRIVED ARTERIES

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Nitric oxide (NO) produced by NO synthase (NOS) in the endothelium in response to vasorelaxants such as acetylcholine (ACh) acts on vascular smooth muscle cells (VSMC) inducing vascular relaxation. However, it was found that VSMC express NOS by themselves, but the principal question remained unanswered, if the NO generation by VSMC is physiologically relevant. We hypothesized that the destruction of the anatomical integrity of arteries by rubbing off the endothelial layer led to a vascular dysfunction and rendered blood vessels insensitive to vasodilators as a consequence of oxidative stress. To test this hypothesis, we examined ACh-induced vasorelaxation under protection against oxidative stress. For functional relaxation studies the isolated thoracic aorta, mesenteric artery and pulmonary artery of male Wistar rats were used. In one part of vessel rings endothelial cells were removed by gently rubbing the intimal surface. The reactivity of anti-NOS (all isoforms) antibody was confirmed by immunohistochemical staining in tissue probes of the thoracic aorta, mesenteric artery and pulmonary artery. In all arterial rings with intact endothelium, ACh (10^{-10}-$3x10^{-5}$ M) produced concentration-dependent relaxation. In contrast, in rings with denuded endothelium ACh-induced relaxation was inhibited. The following pretreatment of denuded rings of thoracic aorta with N-acetyl-L-cysteine NAC (10^{-4} M) as well as tempol ($3x10^{-3}$ M), free radical scavengers, improved relaxation to ACh compared to untreated rings. Similarly, the pretreatment of denuded rings of mesenteric and/or pulmonary arterial rings with tempol ($3x10^{-3}$ M) refreshed the inhibited relaxation to ACh. Immuno-histochemical staining revealed that all three NOS isoforms were expressed not only in the intima but also in media of the blood vessels. Strong expression of NOS3-isoform in all in both intimal and medial cells was proved. VSMC can release NO in amounts sufficient to account for the vasorelaxatory response. Restoration of vasodilatory responses to ACh in endothelium deprived blood vessels under protection against oxidative stress challenges the concept of the exclusive role of endothelial cells in the relaxation of VSMC.

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EFFECT OF ACUTE AND CHRONIC NIFEDIPINE ADMINISTRATION ON THE REACTIVITY OF ISOLATED CONDUIT ARTERIES IN YOUNG SPONTANEOUSLY HYPERTENSIVE RATS

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It is evidenced that in chronic hypertensive state there is an increased vasodepressor response to calcium channel antagonists like the dihydropyridine derivate nifedipine. This effect is generally proportional to initial blood pressure (BP) as was demonstrated in several models of experimental hypertension (1). Furthermore, the increased function of voltage-dependent calcium channels in hypertension seems to be linked to the enhanced tone of sympathetic nervous system, which is also a common denominator of various forms of experimental hypertension. In spontaneously hypertensive rats (SHR), which are anatomically hyperinervated, characteristic acceleration of the BP rise occurs mainly between 4^{th} and 10^{th} week of age and is also accompanied with maturation processes in cardiovascular sympathetic neurotransmission. Here we present our preliminary observations regarding the effect of nifedipine treatment on the cardiovascular system of young SHR to evaluate the possibility to prevent its abnormalities leading to hypertensive state. Rats were treated with nifedipine (50 mg/kg/day) from weaning for 4 weeks (i.e. from 4^{th} to 8^{th} week of life). Blood pressure of nifedipine-treated rats remained at the level of normotensive animals in contrast to their untreated controls in which BP continued to increase. In isolated superior mesenteric arteries, chronic nifedipine treatment significantly reduced noradrenergic contractions elicited by exogenous noradrenaline and by endogenous noradrenaline released from sympathetic nerves during transmural electrical stimulation. This reduction was similar in both types of reaction indicating that nifedipine influences sympathetic vasoconstriction predominantly at the level of vascular smooth muscle. This effect was also seen in experiments with acute nifedipine application on arterial preparations during noradrenergic stimulation by means of transmural electrical stimulation or exogenous noradrenaline. On the base of these results we can presume that chronic reduction of calcium influx may eliminate the effect of enhanced sympathetic tone which may have unfavourable consequences on cardiovascular structure and function.

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INHIBITION ON CARDIOVASCULAR SYSTEM OF SHR AND WISTAR RATS

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We demonstrate long-term effect of neuronal NO-synthase (nNOS) inhibitor 7-nitroindazole (7-NI) on heart, coronary arteries and thoracic aorta of SHR and Wistar rats. Ten weeks old rats were divided in four groups (20 rats in each): Wistar rats, SHR, and Wistar rats and SHR treated by 7-NI (10 mg/kg b.w./day for 6 weeks in tap water). Blood pressure (BP) was measured by the plethysmographic method weekly. For morphological study ten animals of each group were perfused with a glutaraldehyde fixative (120 mm Hg). Coronary artery (CA) and thoracic aorta (TA) were excised and processed for electron microscopy. Tunica intima+media of the vessels were investigated. In ten rats TA was after excision, cut into rings and used for functional study. 7-NI administered to Wistar rats (compared to Wistar rats) did not affect BP but heart/body weight ratio decreased. Hypotrophy of the both vessels was observed (decrease of wall thickness - WT, cross sectional area - CSA, and WT/inner diameter – WT/ID). In CA CSA of both endothelial and smooth muscle cells markedly decreased while CSA of extracellular matrix did not change. Relaxant response of TA to acetylcholine (10^{-9}-10^{-5} mol/l) was moderately inhibited and contraction induced by administration of exogenous noradrenaline (10^{-9}-10^{-5} mol/l) was attenuated. 7-NI in SHR (compared to SHR) did not affect BP but heart/body weight ratio was increased. Morphological investigation of the CA surprisingly revealed hypertrophy of the wall (increased WT, CSA, and WT/ID) as well as increased CSA of endothelial cells, smooth muscle cells, and extracellular matrix. No changes in this respect were observed in TA. Acetylcholine-induced relaxation and/or contraction to exogenous noradrenaline were not affected. In conclusion: Long-term administration of 7-NI to normotensive Wistar rats evoked pressure independent cardiac hypotrophy and vessel wall hypotrophy due to decrease of endothelial and smooth muscle cell mass, which was associated with decreased contractile efficiency. Opposite to Wistar rats, 7-NI administered to SHR had no effect on general hemodynamic parameters and the structural and vasoactive properties of the TA. In the heart and CA 7-NI evoked hypertrophy. Different effect of 7-NI in normotensive and hypertensive rats could be evoked by affecting (i) the different regulatory pathways or (ii) the same pathways which might have different activity in normotension and hypertension.

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Session VI
Diabetes, hypercholesteremia and cardiac function

ADAPTIVE MECHANISMS IN THE DIABETIC MYOCARDIUM: AN ALTERNATIVE FORM OF ENDOGENOUS CARDIOPROTECTION

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High glucose (HG) is one of the factors determining the outcome of ischemia/ reperfusion (I/R) injury, however, it is still not clear whether it is casually related to lower ischemic tolerance in diabetics. Experimental data suggest that besides higher myocardial vulnerability, diabetes mellitus (DM) may induce adaptation leading to paradoxical resistance to I sharing some molecular pathways with endogenous protection (preconditioning) against I/R in non-diseased heart. We addressed the issue of ischemic tolerance in DM heart, its modulation by another pathological state, hypercholesterolemia (HCH), and explored a potential link between cardiac response to I/R and gene expression of PPAR as main transcriptional regulators of lipid metabolism and energy production. Furthermore, we investigated the effects of HG in non-DM hearts considering the involvement of PI3K/Akt in cardioprotective mechanisms. Langendorff-perfused hearts of DM rats (STZ 65 mg/kg, i.p., 1 week) with blood glucose >20 mM and age-matched controls (C) were subjected to 30-min occlusion of the LAD coronary artery/2-h R for the determination of the infarct size (IS, in % of risk area) and PPAR gene expression (RT-PCR) or to 30-min global I/40-min R for the evaluation of postischemic recovery of left ventricular developed pressure (LVDP), respectively. Non-DM hearts were exposed to 5-min HG treatment mimicking DM (glucose 22 mM) with or without application of a PI3K inhibitor wortmannin (100 nM; 20 min prior to I/R). Results: Lower susceptibility to I/R in DM hearts was documented by a 2-fold higher LVDP recovery, reduction of IS by 60 % ($P<0.05$ vs. C) associated with preservation of baseline mRNA levels of PPAR in contrast to their downregulation in C. HCH induced by simultaneous fat-cholesterol diet in diabetics blunted both, cardioprotection and changes in PPAR levels. In the non-diabetic hearts, HG treatment conferred an efficient protection similar to that in the DM heart that was completely abolished by wortmannin. Conclusions: DM might induce adaptive processes in the heart associated with the changes in PPAR gene expression. HG may contribute to the mechanisms of enhanced ischemic tolerance via activation of PI3K/Akt. Protective mechanisms activated in DM may be suppressed by concomitant pathology.

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EFFECTS OF HYPOLIPIDEMIC DRUGS SIMVASTATIN AND PRAVASTATIN ON ISCHEMIC-REPERFUSION INJURY IN ISOLATED RAT HEART

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Hypercholesterolemia is one of the major determinants of coronary artery disease (CAD) as the leading cause of death worldwide. Statins, the inhibitors of HMG-CoA reductase, are frequently used in prevention of CAD due to decrease of plasma cholesterol level. However, inhibition of this enzyme may provide beneficial effects beyond cholesterol-lowering (1). Pleiotropic actions of statins have been found to suppress inflammation, oxidative stress, thrombogenesis and atherosclerotic plaque formation. Mechanisms of this protection include increased endothelial NO synthase activity and a subsequent rise in NO bioavailability. The aim of our study was to compare the effects of chronic treatment with simvastatin (S) and acute application of pravastatin (P) on myocardial I/R injury. After 5-day treatment with S (10 mg/kg/day, p.o.), Langendorff-perfused hearts were subjected to 30-min regional (occlusion of the LAD coronary artery) or global ischemia/2-h reperfusion for the evaluation of the infarct size (IS, in % of area of risk, AR) measured by planimetry and postischemic recovery of left ventricle developed pressure (LVDP) and coronary flow (expressed in % of preischemic values). Pravastatin (P) was given as a component of perfusion buffer (30 μmol/l), 15 min before 30-min global ischemia and 2-h reperfusion. Results: In S-treated group, recovery of mechanical function was increased from 24.1±2.9 % in the untreated controls (C) to 76.1±9.8 % ($P<0.05$). Likewise, infarct size was reduced (IS/AR 11.5±0.4 % vs. 33.7±4 in C; $P<0.05$). On the contrary, P did not change postischemic recovery of LVDP (16.09±5.2 % vs. 22.7±3 % in C; $P>0.05$), however, IS in P-treated group was significantly smaller than in C group. Both drugs markedly improved coronary perfusion of the myocardium during reperfusion. Since effects of statins were investigated in rats with normal plasma cholesterol profiles, it is obvious that other effects of statins independent of cholesterol-lowering are involved in cardioprotection. Our results further suggest that pleiotropic effects of statins depend on the application mode and different chemical properties of given statins (2,3).

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MYOCARDIAL REMODELING INDUCED BY REPEATED ACTIVATION OF ADRENERGIC SYSTEM

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Increased hemodynamic overload could be observed in relation to increased activity of adrenergic system characterized by positive chronotropic and inotropic effects on the heart. Repeated stimulation of alfa and beta receptors triggers a cascade of adaptive reaction demonstrated as myocardial hypertrophy, however prolonged stimulation leads to exhaustion of neurohumoral activation, and finally to maladaptive electrical and structural events described as a heart failure (HF). Development of left ventricular hypertrophy increases the risk of sudden death. As a predictor of mortality is usually accepted prolonged QT interval. We focused on the effect of repeated application of isoproterenol (Iso) on the electrical activity of rat heart. Electrocardiograms (ECG) were recorded from animals under general anesthesia (thiopental 45 mg/kg) as well as from the isolated spontaneously beating perfused hearts according to the Langendorff. Myocardial remodeling in rats was induced by repeated injections of isoproterenol (5 mg/kg s.c.) either for 7 days (Iso5/7, n=7) or for 21 days (Iso5/21, n=5). Voltage criteria e.g. Sokolow-Lyon indexes were decreased in the order Iso5/7 > Iso5/21 > baseline criteria found in control (n=5) group as well as the thickness of the free ventricular wall. However, heart and left ventricular wet weight, as well as the Cornell index were increased with longer Iso application compared to controls. Prolonged QT interval found in Iso modified animals increased susceptibility to ventricular arrhythmias. Hemodynamic measurements of Iso5/7 hearts revealed weaker contractile ability normalized to 1 g myocardial mass (61±3 vs 97±5 mmHg/1 g) of spontaneously beating hearts compared to control hearts but the weakest contraction was observed in the Iso5/21 (32±4 mmHg/1 g). Mortality of Iso5/7 rats was about 40-50 % and increased with longer Iso application to cca 75-85 %. Tissue examination showed in Iso5/7 focal necrotic loci in the left ventricle but extended areas of necrosis in the endocardium in Iso5/21. It could be concluded that repeated doses of isoproterenol induce electrical remodeling associated with hypertrophy, however, longer application could result into the failing myocardium with increased incidence of arrhythmias and sudden death.

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ACUTE DIABETES MELLITUS AND ITS INFLUENCE ON RENAL Na,K-ATPase IN BOTH GENDERS

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Due to the importance of renal Na,K-ATPase in maintaining the sodium homeostasis in the organism, its activity and abundance is intensively studied in condition of diabetes mellitus. The main subject of this study was the investigation of properties of renal Na,K-ATPase and abundance of its alpha1 subunit in view of possible gender-dependent differences in male and female diabetic rats. Diabetes was induced by a single intraperitoneal dose of streptozotocin in a dose of 65 $mg.kg^{-1}$. The acute diabetes lasting 8 days induced a significant increase in Na,K-ATPase activity accompanied by significant gender specific increase in K_m value indicating a worsened affinity of ATP-binding site in female rats. In addition, our present experiments, revealed a significantly higher abundance of renal Na,K-ATPase alpha1 subunit in diabetic rats of both genders amounting 94 % increase in males and 107 % in females. But, not all of the newly synthesized enzyme molecules are fully active, as the increase in the number of active molecules is smaller (representing 23 % in males and 20 % in females) as indicated by lower increase in V_{max} values.

THE EFFECT OF NOVEL PYRIDOINDOL DERIVATIVE SMe1EC2 AND ATORVASTATIN ON SELECTED PARAMETERS IN HEREDITARY HYPERTRIGLYCERIDEMIC RATS FED HIGH-CHOLESTEROL DIET

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Elevated plasma cholesterol, especially low density lipoprotein (LDL) cholesterol, is one of the major risk factors for atherosclerosis and coronary heart disease. Hereditary hypertriglyceridemic rats (hHTG) were developed as a new inbred model for the study of relationships between blood pressure and metabolic abnormalities (1). The aim of this work was to determine the cholesterol lowering and antioxidant effects of the novel pyridoindol derivative SMe1EC2, compared to the cholesterol-lowering drug, atorvastatin, in rats fed either control or high-cholesterol diet (hCholD; 1 % cholesterol and 7.5 % lard fat). Male hHTG groups of rats fed a hCholD were administered with SMe1EC2 (30 mg/kg/day p.o.) or atorvastatin (50 mg/kg/day p.o.) for 4 weeks, and compared with groups of rats fed standard diets. Physiological status of animals was monitored by the measurement of preprandial glucose level and invasive blood pressure. Lipid profile was characterized by the serum levels of total cholesterol (TC), HDL-, LDL-cholesterol and triglycerides (TRG). Others parameters evaluated were TBARs in serum and the kidney, lysosomal enzyme N-acetyl-ß-D-glucosaminidase

(NAGA) in serum and the kidney. To investigate whether inflammatory parameters might play role in pathogenesis of hypercholesterolemia and hypolipidemic protective effects of tested compounds, the assessment of pro-inflammatory cytokines TNF-α, IL-1 and IL-6 in the serum was completed. Feeding the animals hCholD resulted in marked hypercholesterolemia and increased the serum level of TC, LDL-C and TRG. Pyridoindol tested, SMe1EC2, affected selected parameters in hHTG rats, especially those related to lipid profile: decreased serum levels of TC and TRG in hHTG rats, either on control or hCholD diet. In conclusions, the results of current study showed that SMe1EC2 was able to lower the levels of lipid parameters, similarly as atorvastatin, the standard hypolipidemic drug.

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THE EFFECT OF SMe1EC2 AND FENOFIBRATE ON SELECTED PARAMETERS IN HEREDITARY HYPERTRIGLYCERIDEMIC RATS FED HIGH-CHOLESTEROL DIET

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Metabolic syndrome (MetS) is regarded as a cluster of related risk factors for cardiovascular disease, type 2 diabetes and liver disease. To mimic MetS and to resemble human hypertriglyceridemia, the model of Prague hereditary hypertriglyceridemic (hHTG) rats was established with following symptoms: not obese but hypertensive rats, insulin resistant with some disturbances in glucose metabolism (1). The purpose of this study was i) to adapt such a model in our conditions; ii) to study the effect of long-term administration of pyridoindol SMe1EC2 (10 mg/kg/day *p.o.*) on selected parameters in hHTG rats fed either control or high-cholesterol diet (hCholD – 1 % cholesterol + 7,5 % lard fat); iii) to compare SMe1EC2 actions with those of fenofibrate (100 mg/kg/day *p.o.*). Physiological status of animals was monitored by the evaluation of preprandial glucose level, non-invasive measured blood pressure and oral glucose tolerance test. Lipid profile was characterized by the serum levels of total cholesterol (TC), HDL-, LDL-cholesterol and triglycerides (TRG). Others parameters followed were creatinine in serum and urine, TBARs in serum and the kidney, lysosomal enzyme N-acetyl-ß-D-glucosaminidase (NAGA) in serum, urine and the kidney. Moreover, serum levels of cytokines IL1, IL6 and TNF-α were also assessed. At the end week 4th of experiment, hHTG rats had significantly elevated levels of TC and TRG, as compared to age-matched Wistar rats. However, compared with the previous study (1), neither impaired glucose tolerance nor elevated blood pressure in hHTG rats were validated. Novel compound tested, SMe1EC2, similarly as clinically used fenofibrate, affected selected parameters in hHTG rats, especially those related to lipid profile: decreased level of TC and TRG in hHTG rats, either on control or hCholD diet. These findings render SMe1EC2 to promising agents with perspective in further surveys related to MetS features.

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Session VII
Nitric oxide in pathological processes

NITRIC OXIDE SYNTHASES EFFECT ON APOPTOSIS ACTIVATION IN THYROID GLAND AUTOIMMUNE DISEASES

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Nitric oxide might influence the development of various pathological processes in the organisms. The presented study evaluated the changes of NO synthase expression in thyroid gland diseases in relation to the changes of selected cell cycle markers. Both, the inducibile and the endothelial nitric oxide synthase showed similar expression pattern in autoimmune thyroid gland diseases. Hashimoto disease showed cytoplasmic both NOS isoforms expression, in Graves-Basedow goiter there was strictly nuclear expression of eNOS and also with cytoplasmic positivity of iNOS. The proapoptotic factors BAX, caspase 3 and APAF and the antiapoptotic survivin were increased in both types of diseases. The antiapoptotic Bcl2, p53 and PCNA expression dominated in Hashimoto disease. The results indicate, that NO may be a factor involved in the development of the autoimmune thyroiditis. It can be hypothesized that the iNOS as well as the eNOS nuclear translocation might interfere with apoptosis activation in these diseases and thus can play a role in neoplastic transformation process in the thyroid gland tissue.

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NITRIC OXIDE – MEDIATOR OR MARKER OF NEOPLASTIC TRANSFORMATION?

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Nitric oxide plays an important role in the development of various pathological states in the organism. Up to the presence, a number of studies has been trying to elucidate the spectrum of its signaling functions from cardiovascular system to cancerogenesis (1). The presented study evaluated the nitric oxide synthase NOS2 and NOS3 expression by immunohistochemistry in various tumors of the thyroid gland. The archival bioptic samples, included the cases of parenchymatous goiter, benign follicular adenoma, malignant follicular, papillary, follicular variant of papillary, medullary and anaplastic carcinoma were processed in tissue microarray. The results were correlated by Western-blot analysis. In comparison to control parenchymatous goiters, significant increase of NOS2 expression could be found in microfollicular adenoma and malignant well-differentiated carcinomas of the thyroid gland. NOS3 expression was the highest in oncocytic carcinoma, moderate in follicular carcinoma and just inconsistent in papillary carcinoma. NOS3 was weakly but constantly expressed in blood vessels in all samples. NOS2 was in the past described to play a role in carcinogenesis as the mediator of DNA damage, in neovascularisation and vasodilatation, which can support the tumor growth. The increased NOS2 expression in the thyroid gland tumors supports this theory. The pharmacological inhibition of NO production was shown to be beneficial in the cancer therapy (2). However, the exact role of NO in tumorigenesis is controversial, some studies showed that the induction of NOS2 can activate the protective pathways in affected cells and can lead to cell apoptosis and tumor cell regression implying that NO could be a perspective target in the anticancer therapy. The presented findings should be taken in consideration also when the therapy affecting NO production is administered to humans for other reasons, especially cardiovascular diseases.

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ROLE OF NITRIC OXIDE IN DEVELOPMENT AND PROGRESSION OF MULTIPLE MYELOMA

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Nitric oxide (NO) plays an important regulatory role in several biological processes under physiological and pathological conditions. Increased NO synthesis has been documented in a variety of neoplasms, including hematological malignancies (1), but the biological function of NO in tumors is still controversial. Whereas on one hand NO could act as an antitumorous agent with proapoptotic and cytotoxic effects (2), on the other hand it could cooperate in cancerogenesis initiation and progression, as well as in processes of angiogenesis. Recent studies indicate that beside solid tumors, angiogenesis is an important prognostic factor in hematological malignancies, especially in multiple myelomas, where the increased density of capillaries seems to correlate with shorter survival of patients (3). The presented study evaluated the changes of NO synthase isoform expression in bone marrow biopsies from patients with multiple myeloma (n=15) and normal or reactive bone marrow samples (n=5). Both, tumor and control samples showed positivity of all NOS isoforms. No significant changes in i-NOS and n-NOS expression were found. e-NOS demonstrated cytoplasmic positivity pattern in endothelial cells, granulocytes and megakaryocytes. Multiple myeloma samples showed increased e-NOS expression compared to control samples ($p<0,01$). This increased positivity of e-NOS corresponded to higher density of blood vessels in these malignancies demonstrated by increased PECAM-1 expression. Our results suggest that plasma cell neoplasms are able to express immunohistochemically detectable NOS. Particularly e-NOS seems to play an important role in the production of NO in multiple myeloma (1), however its role in the biology and prognosis of this neoplasm remains to be established.

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NATURAL COMPOUNDS ADMINISTRATION IN PREVENTION OF ACUTE TOXIC LIVER INJURY

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Free radical injury can play a causative role in chronic as well as in acute liver injury (1). Polyphenolic compounds, rooibos tee and red wine polyphenols included, act as efficient scavengers of reactive oxygen species (2) and can also influence NO production through increased NO synthase (NOS) activity (3). NO is one of the key factors important for liver recovery and protection. The aim of this study was to evaluate the effects of polyphenolic compounds in the development of acute liver damage induced by carbon tetrachloride or ethanol administration. Wistar rats were divided into 9 groups: control group, groups receiving CCl_4 intraperitoneally *(1 ml/kg)* or ethanol through intragastric gavage during 3 days of intoxication and groups receiving during 5 days before the intoxication red wine extract (*30 mg/kg/day*) or rooibos tee instead of water supply. Ethanol administration led to centrolobular vacuolar degeneration of the hepatocytes, whereas carbon tetrachloride caused liver steatosis development. Both, red wine polyphenols and rooibos tee prevented these changes. The decreased synthesis of iNOS was found in both, CCl_4 and ethanol receiving animals. Cytoplasmic expression of iNOS was localized in controls mostly in the pericentral areas, whereas in CCl_4 treated animals only weak positivity in regions of steatosis was detected. Polyphenolic compounds administration led to the increase of iNOS expression to the control levels. eNOS expression has not shown any significant changes. In conclusion, both polyphenolic compounds used in this experiment demonstrated protective effect in acute liver injury. These effects may be due to radical scavenging properties or the influence on NO production.

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DUAL EFFECT OF ROOIBOS TEA TREATMENT IN CHRONIC LIVER INJURY

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Oxidative stress appears to play an important role in chronic liver disease development. Rooibos tee contains a family of polyphenolic compounds, which have shown their beneficial effects in several pathological states (1). The presented study evaluates the effects of rooibos tea on the development of experimental chronic liver damage induced by carbon tetrachloride administration. Male Wistar rats were divided into 4 groups: control group, group receiving rooibos tea instead of drinking water, group receiving carbon tetrachloride intraperitoneally (1 ml/kg) during 10 weeks with following 6 weeks of spontaneous recovery and group receiving both carbon tetrachloride and rooibos tea during intoxication and recovery phase. Administration of carbon tetrachloride was accompanied by increased levels of malondialdehyde and liver injury markers, and led to the development of liver steatosis and fibrosis. Rooibos tee demonstrated a protective effect and reduced fibrosis in the liver. In contrast liver steatosis was more pronounced in rooibos drinking animals, compared to all other groups. The rooibos tea treatment caused also the decrease of Apaf, NFkB and NOS2 expression in the liver tissue, compared with the animals without the rooibos tea treatment. NOS2 was shown to be important factor in liver regeneration (2). Inhibition of NOS2 expression caused by rooibos tea can explain the increased liver steatosis during the recovery. It can be concluded, that when administered during intoxication phase, rooibos treatment prevents the development of serious liver damage. However, during the recovery phase it may lead to prolonged liver regeneration. The dual effect of strong antioxidants should be taken in consideration when used in therapeutic implications.

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CURCUMIN AND PIPERINE IN L-NAME INDUCED HYPERTENSION – ROLE OF INDUCIBLE NO SYNTHASE

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The effects of curcumin and piperine on blood pressure were studied in the model of N(G)-nitro-L-arginine methyl ester (L-NAME)-induced experimental hypertension. Adult male Wistar rats were given curcumin (100 mg/kg/day), piperine (20 mg/kg/day) or their combination in corn oil by gavage, with or without L-NAME (40 mg/kg/day). Aorta cross-sections were stained by phosphotungstic acid hematoxylin, orcein and with antibodies against inducible NO synthase (iNOS). The already low expression of iNOS protein in aortic media of control animals (4,6 % of the cross sectional area) was further lowered by L-NAME (0,5 %). Piperine mildly prevented the elevation of blood pressure, although it did not affect the smooth muscle, elastin or iNOS content in the media. Curcumin significantly lowered the blood pressure and fully prevented the increase of smooth muscle mass, decrease of elastin and iNOS in the aortic media induced by L-NAME in hypertensive animals. Surprisingly, curcumin alone increased the iNOS content in the media. The combination of spices caused a non-significant decrease of blood pressure, but the effects on aortic media were analogous to those caused by curcumin alone. The impact of curcumin on smooth muscle cells can be explained by its well known antiproliferative properties (1) that are probably enhanced by the increased iNOS content (2). Piperine probably influences the blood pressure by some central mechanism (3) which seems to be more important on the blood pressure than the peripheral effects of curcumin on the aortic media.

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THE STUDY OF REACTIVE NITROGEN SPECIES IN PLANTS EXPOSED TO STRESS FACTORS TREATMENT

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Plants are frequently challenged by potential biotic and abiotic stressors and have therefore evolved inducible defense mechanisms to survive in their environment. A number of signaling molecules participate in the transfer of information about stress factor action and subsequent initiation of defense responses. Reactive oxygen species (ROS) and recently intensively studied nitric oxide (NO) play a significant role. NO is a widespread intracellular and intercellular messenger with a broad spectrum of regulatory functions in many physiological and pathological processes. In contrast to the large body of knowledge on functions of NO in animal systems, little is known in plant systems. NO emission from plant was first observed by Klepper in 1975. Recent studies have suggested that NO acts as an important effector of seed and pollen germination, plant growth, development, opening and closure of stomata and initiation of defense responses elicited by stress factors treatment. The most published work has focused on the role of NO in the initiation or propagation of the plant hypersensitive response, programmed cell death occurring at the site of infection. A number of possible enzymatic sources for NO have been proposed along with nonenzymatic mechanisms. There are several enzymes that have been shown to produce NO in plants, such as nitrate reductase, xanthine oxidase, NO synthase, nitrite:NO reductase or horseradish peroxidase. The aim of our work is to study the role of reactive nitrogen species in plant reaction to stress elicited by the biotrophic pathogen infection, temperature changes, increasing salt concentration (salinity stress), high mannitol concentration (osmotic stress), toxic metals and by the pathogen infection after the short-time exposure of plants to heat or cold shock. The main model system used for the realization of experiments focused on the study of plant reaction during pathogenesis are especially three *Lycopersicon* spp. species differing in the level of resistance to *Oidium neolycopersici* infection and also *Lactuca* spp. infected by the pathogen *Bremia lactucae.*

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Session VIII
NO signaling and nervous system

NITRIC OXIDE INFLUENCES HUMAN HIGHER NERVOUS REGULATIONS

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It has been accepted that nitric oxide (NO) release within the brain is necessary for induction of long-term potentiation which involves the electrophysiological events related to synaptic plasticity and learning. The NO synthase inhibitors disrupt the performance of animals in learning tasks while the NO donors display an antiamnestic action. The study comparing the effects of L-NAME and molsidomine upon the memory tasks in rat pointed to the fact that the NO is involved in post-training memory processes (1). The possibility that NO may influence also the human higher nervous regulations was studied by analyzing the effect of NO upon the sensorimotor interactions. The visual-oculomotor interactions represent the most subtle as well as most precise kind of the sensorimotor interactions. It was the reason for selecting the saccadic eye movements for analyzing the probable effect of NO synthase activator, Provinols™, administration upon the visual perception and space memory functions. Saccades – the rapid movements of the eyes by means of which subject scans the visual environment – may be elicited by visual stimuli and intentional effort as well. For this study the analysis of the accuracy of visually evoked saccades and of saccadic eye movements driven by memory information about desired eye landing position in space was applied. With every healthy volunteer the saccades elicited by the visual targets were followed by the memory-guided saccade task. The whole experimental procedure was repeated 2 hours following the Provinols™ administration (4 mg/kg of body weight). According to the clock face the saccadic landing positions were randomly indicated at 1, 2, 4, 5, 7, 8, 10 and 11 hour. The Provinols™ application positively affected the accuracy of both the saccades. The memory-guided saccade task comprises 3 phases: 1. perception, 2. memorization and 3. execution of a saccade. Perception is under control of the network which sub serve also the attention mechanisms. Memorization is controlled by the dorsolateral prefrontal cortex and programming and generation of a MGS involve the working space memory. The question which of the above mechanisms is affected by the Provinols™ administration is discussed. Different significance of vertical and horizontal visual half-fields was taken into account.

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A CONTRIBUTION TO THE STUDY OF THE RELATIONSHIP BETWEEN NO SIGNALING AND ATTENTIONAL MECHANISMS

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A fundamental goal of cognitive neuroscience is to identify the neural substrates of higher-order cognitive processes including attention, perception, language and memory. The brain is capable of carrying out multiple information processes simultaneously, but the capacity of our consciousness is limited and therefore must concentrate on part of the information process. This selection mechanism is called attention. Sustained attention represents a basic attentional function that determinates the efficacy of the higher aspects of attention (selective and divided attention) and of cognitive capacity in general. Human

neuropsychological and functional imaging studies have pointed to fronto-parietal areas, particularly in the right hemisphere, as being prominently involved in the mediation of sustained attention, or vigilance which are independent on the visual, auditory and somatosensory modality. The attentional mechanisms are closely related to the eye movement search and/or fixation. The shift of visual attention activates the same neuronal networks as those of programming the saccadic movements of the eyes. The saccadic eye movement related potentials (SEMRPs) may be used in order to add knowledge on the attentional mechanisms. In a previous contribution we have pointed to the fact that the nitric oxide (NO) synthase activator Provinols™ affected the accuracy of the visually and memory guided saccades. In an another examination of healthy volunteers we found out that the programming and execution of visually evoked saccades as well as the primary encoding of new visual information are delayed in an attention distraction task. Also the timing of the successive rapid eye movements depends on the divided attention task. The fronto-parietal interconnections via thalamus are important for the orienting the attention, particularly for disengagement, shifting and engagement of attentional focus. The thalamic pulvinar is of particular importance while it plays a role in release of acetylcholine and NO. It was shown that this release is mirroring the behavioral arousal state. Our results may be of interest because the proposal that the NO synthase activators can enhance memory by improving cerebral blood flow has not been supported. The hypothesis concerning the changes of the NO-producing neurons during learning and memory may be accepted and the attention may be denoted as a “Gate” to learning and memory.

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