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VASODILATORY RESPONSES UNDER HYPOXIC CONDITION: ROLE OF PGI_2 AND NO

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Aim of the present study was to test hypothesis that low availability of oxygen in hypoxic tissue should inhibit oxygenation of arachidonic acid and thereby result in inhibition of eicosanoid synthesis *in vivo*. Perfusion of the isolated rabbit ear with normoxic or hypoxic medium was applied. Eicosanoid biosynthesis and functional responses of the vascular bed were followed. Simultaneous recording of prostaglandin I_2 release and peripheral resistance of the preparation revealed that lack of oxygen 1) inhibited arachidonate oxygenation, and 2) converted the vasodilatory response to vasoconstriction. Responses were induced by ionophore A23187 (10 nmol in a 10-µl bolus). Cyclooxygenase-1 (COX-) inhibitor (SC560, 1 µmol/l) effectively inhibited normoxic prostaglandin I_2 biosynthesis, while COX-2 inhibitor (DFU, 1 µmol/l) was ineffective. On the other hand, neither COX-1 inhibition nor COX-2 inhibition did affect vasodilatory reactions under normoxia suggesting no involvement of prostanoids in normoxic vasodilatation. In addition, application of nitric oxide synthase inhibitor (L-NAME, 100 µmol/l) converted the evoked vasodilatation reaction into normoxic vasoconstriction. Taken together, our findings indicate that NO-mediated vasorelaxation could be removed during hypoxia by the inhibition of NO synthase due to lack of oxygen similarly as the hypoxic inhibition of arachidonate oxygenation did.

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THE EFFECT OF CHRONIC NOS INHIBITION ON ALTERATIONS OF REGULATORY PROTEINS IN RAT HEARTS

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Nitric oxide (NO) can potentially exert either benificial or deleterious effects in pathological situations and has been implicated in the mechanisms of cardiac adaptation to ischemia. Regulatory proteins and intracellular signaling pathways could play an important role in myocardial adaptive responses. Our aim was to characterize the effects of chronic NOS inhibition by L-NAME treatment on the modulation of ischemic tolerance and on the alterations of regulatory myocardial proteins at the subcellular level. Chronic NO deficiency (NOD) was induced by L-NAME (40mg/kg/day, 4 weeks). Isolated hearts from control rats or rats with NOD were Langendorff-perfused and subjected to test index ischemia induced by 25 min global ischemia and 35 min reperfusion (IR). Tissue samples were taken from the left ventricles and protein fractions were prepared as described previously (1). Protein levels and phosphorylation of specific proteins were determined by Western blot analysis.

Activities of matrix metalloproteinases (MMP) were analyzed by zymography in polyacrylamide gels containing gelatine as a substrate. Development of NOD was connected with decreased activation of extracellular signal-regulated protein kinases (ERK) and eNOS and the levels of upstream activators of ERK (aFGF, H-Ras) were also decreased. The hearts from rats with NOD showed better recovery of contractile function after IR. During IR the ERKs were activated and the activation was further increased in NOD hearts. In addition, decrease in activities of tissue MMP-2 was found in these hearts. On the other hand, in serum of L-NAME treated rats significantly increased gelatinolytic activity of approxim. 20 kDa proteinase was observed.

The effects of NOD on components of ERK signaling pathway, the changes in ERK activation during IR and the effects of NOD on this activation suggest the involvement of this signaling pathway in responses of myocardium to NOD and in adaptive responses of these hearts to ischemic stress. The results point also to the possible relationship between ERK pathway and activation of eNOS and/or tissue MMP-2.

1. Strohm et. al., J. Cardiovasc. Pharmacol. 2000, 36:218-229

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DUAL ROLE OF NO IN SUSCEPTIBILITY TO ISCHEMIA/ REPERFUSION INJURY IN THE RAT HEART

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In pathological situations (e.g., ischemia/reperfusion, IR), nitric oxide (NO) can potentially exert either beneficial or deleterious effects, and NO function differs in the intact heart and in the myocardium adapted to short-term and long-lasting stressful stimuli. In a setting of test IR (TI) in the Langendorff-perfused rat heart, acute blockade of NO production by NO synthase (NOS) inhibitor L-NAME improved postischemic recovery of LVDP and suppresed severe ventricular arrhythmias induced by global IR in non-adapted hearts (arrhythmias score $2,7 \pm 0,3$ vs $3,8 \pm 0,2$ in the controls; $p<0,05$). In addition, the detrimental role of NO was supported by limitation of infarct size (TTC staining) normalized to the size of area at risk (IS/AR) from $42 \pm 5,1$ % in the controls to $28,4 \pm 1,6$ % in the L-NAME-treated hearts ($p<0,05$). On the other hand, L-NAME treatment abrogated antiarrhythmic and infarct-limiting effects in the heart subjected to ischemic preconditioning (IP) by two brief episodes of IR, prior to IT. Oxidative load is a common feature of a number of chronic processes associated with heart remodeling on one hand, and development of long-term adaptation on the other hand. In chronic L-NAME-treated rats, the heart from hypertensive animals appeared to be less susceptibile to I/R injury in a phase of compensated hypertrophy. Similary to IP, protective effect was associated with enhanced ERK1/2 activity during I/R and was reversed by both, antioxidant NAC and mito K(ATP) blocker 5-HD. It is concluded that acute blockade of NO production might be beneficial in the intact myocardium exposed to prolonged I/R. On the other hand, NO might be also implicated in the mechanisms of short-term and long-term cardiac adaptation.

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THE ROLE OF FREE RADICALS AND MITOCHONDRIAL K(ATP) CHANNEL MODULATIONS IN THE MECHANISMS OF SHORT-TERM CARDIOPROTECTION IN THE RAT HEART

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Myocardial ischemia/reperfusion (I/R) results in a burst of free radicals (FR) including those derived from O_2 and NO traditionally viewed as cardiotoxic. Although FR have not been initially considered as signaling molecules, fluctuations in the oxidant/ antioxidant balance are now recognized as an important regulator of a variety of cellular functions in health and disease (energy production, survival kinases activation, apoptosis signaling, necrosis, oxygen sensing, etc.). The function of FR differs in the unstressed and adapted myocardium and their relationship with mitochondrial K(ATP) channels opening is not completely clear. Our aims were to examine the role of FR and their relationship with mitochondrial K(ATP) channels [(mK(ATP)] in the Langendorff-perfused rat hearts subjected to test ischaemic challenge (TI) with or without preconditioning (PC). Ischaemic PC or K(ATP) opening by diazoxide (D) increased production of FR as evidenced by an increased

concentration of conjugated dienes (CD) in the myocardium prior to the onset of TI, and ameliorated I/R injury (improved postischaemic recovery of LVDP and reduced arrhythmogenesis). Moreover, IPC suppressed enhanced production of FR in the heart during subsequent TI. Application of antioxidant (N-acetylcystein, NAC) in the intact hearts also normalized elevation of CD during TI, as well as attenuated I/R injury. In the hearts preconditioned before TI, pretreatment with NAC or mK(ATP) blocker 5-HD reduced production of FR during prolonged ischaemia, however, both interventions abrogated protective effects of PC on postischaemic recovery. It is concluded that FR might play a dual role in I/R injury: being deleterious in the intact non-adapted myocardium exposed to prolonged ischaemia, they might be also implicated in cardioprotection conferred by a brief ischaemia and/or mK(ATP) opening.

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CONTINUOUS LIGHT EXPOSURE PROTECTS RAT HEART AGAINST ISCHEMIA-REPERFUSION INJURY: THE ROLE OF NO-SYNTHASE

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Ischemic heart disease is one of the leading causes of mortality. Thus the mechanism(s) of preconditioning aimed to decrease the myocardial susceptibility to ischemia-reperfusion (I/R) injury still represents an actual cardiologic problem. Long-term NO-synthase (NOS) inhibition was demonstrated to precondition the heart to I/R injury. In our study, we aimed to investigate, whether continuous light exposure may modify the susceptibility of rat heart to I/R injury via the modulation of NOS pathway. Two groups of male adult Wistar rats were investigated: control (12/12 light/dark cycle, n=22) and light-exposed (24 h light for 4 weeks, n=23) group. Perfused isolated hearts (Langendorff technique) were exposed to 25 min ischemia and subsequent 30 min reperfusion. The recovery of coronary flow, left ventricular developed pressure, +dP/dt max and -dP/dt max were evaluated in the 15^{th}, 20^{th}, 25^{th} and 30^{th} min of reperfusion. The NOS activity in brainstems and hearts was determined by measuring the conversion of radioactive L-arginine to L-citrulline. NOS expression in brainstems was investigated using Western blot. The post-ischemic coronary flow, contractility and relaxation were improved better in the light-exposed group than in the control group. The NOS activity in brainstems and hearts was decreased in the light-exposed group. The decreased NOS activity was associated with modulated NOS expression. To conclude, the continuous light exposure exerted preconditioning-like effect, possibly due to decreased NO-synthase activity.

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POSSIBLE ROLE OF NITRIC OXIDE SYNTHASE IN REGULATION OF AEROBIC METABOLISM OF THE DIABETIC HEART

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Depression in mitochondrial (MIT) state 3 & 4 QO_2, RCI and phosphorylation rate and CoQ_{10} oxidation ($p<0.05$-0.001) observed in acute diabetic (DIA) hearts may be attributed to reactive oxygen species (ROS) generation in the respiratory chain. But, DIA-induced alterations in citric acid cycle function can not be excluded as participants in the above perturbations either. However, in the acute phase of DIA, the noxious effect of ROS on the heart MIT proved not to be too strong (enhancement in conjugated dienes formation is missing, MIT membrane fluidity is increased ($p<0.05$)). Moreover, the decreased oxid/phosp capacity seems to be, at least in part, compensated by increased MIT Mg-ATPase activity and augmented energy delivery to cytoplasm, via elevated formation of energy permeability transition pores in MIT membranes (all $p<0.05$). Nevertheless, in similar conditions, considerably increased NO and conjugated dienes formation was also reported in heart tissue. Hence, the outcome of metabolic alterations and also of endogenous protective mechanisms (EPM) seems to be organelle-specific. Main objective of the present study is investigation of the role of heart MIT NO-synthase (NOS) in acute phase of DIA. Acute (8 days) DIA in male Wistar rats (220±20 g) was induced by a single dose of streptozotocin (55 mg/kg i.p.). Membrane fluidity (MF) and potential (MP) of isolated MIT (with protease) were assessed by measuring fluorescence anisotropy of DPH (1,6-diphenyl-1,3,5-hexatriene) and by confocal microscopy using JC-1(5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzimidazolylcarbocyanine iodide) as fluorescent sensor respectively. Total MIT NOS activity was estimated by conversion of [^{3}H]-arginine to [3H]-citrulline, NFkapaB and eNOS protein expression by immunoblotting. DIA heart MIT exhibited increased MF ($p<0.01$), decreased MP ($p<0.001$) coupled with enhanced capability to maintain MP, no increases in conjugated dienes formation in membrane lipids and expression of MIT eNOS. Linear regression analysis revealed significant association ($r=0.67$; $p<0.05$) between the increase in MF and decrease in MP in DIA MIT. Elevated content of NFkapaB was also found in the MIT fraction. The total MIT NOS activity was decreased ($p<0.006$) indicating that a considerable part of enzyme molecules might form oxygen producing monomeres. In conclusion, the DIA–induced attack of ROS to heart MIT in acute DIA seems to remain restricted to significant, but not critical damage to electron-oxygen transport and oxid/phosph kinetics and capacity. A probable link between ROS action and depression of total MIT NOS activity, particularly in respect to mono/dimeric organisation of the enzyme can not be excluded, but the whole phenomenon still needs careful investigation. Results point to expressed organelle-specificity of the DIA-induced alterations as well as of the EPM.

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RESPIRATORY CHAIN FUNCTION OF BRAIN AND SKELETAL MUSCLE MITOCHONDRIA IN GENETIC MODEL OF HYPERTENSION

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Nitric oxide (NO) is known to regulate mitochondrial respiration and ATP production by reversible binding to complex IV of respiratory chain (RC) – cytochrome oxidase (1). However, prolonged exposure of mitochondria to elevated concentrations of NO could result in decreased activities of RC complexes I, III and IV (2, 3). Aim of our study was to examine function of RC of brain and skeletal muscle mitochondria in animal model of genetic hypertension, which is known by increased NO synthase activity as well as increased production of reactive oxygen species (4). Experiments were performed on adult Wistar (W) and spontaneously hypertensive rats (SHR). The function of RC of isolated mitochondria in the presence of glutamate or succinate/rotenone as substrate and specific cytochrome oxidase activity were assessed polarographically. Coenzyme Q_9 and Q_{10} (CoQ_9, CoQ_{10}) content in the mitochondria was measured using HPLC method. Results: In the brain

mitochondria of SHR the glutamate-supported respiration and the rate of ATP production were decreased (-24.5 %, $p < 0.001$ for S_3; -19.2 %, $p < 0.005$ for S_4; - 30.04 %, $p < 0.002$ for OPR), and mitochondrial levels of CoQ_9 and CoQ_{10} were diminished (-39 %, $p < 0.015$, and - 47 %, $p < 0.002$).The succinate-supported function and complex IV activity were not affected by hypertension. On the other hand, in skeletal muscle mitochondria of SHR the glutamate- and succinate-supported respiration and the rate of ATP production as well as CoQ_9 concentration was similar to W rats, but the activity of cytochrome oxidase was significantly decreased (-39 %, $p < 0.001$). Conclusion: The sites of respiratory chain impairment in this model of genetic hypertension are different in brain and skeletal muscle mitochondria. The impairment of energy production and the decrease of lipid-soluble antioxidant CoQ concentration in brain mitochondria could contribute to increased vulnerability of brain tissue in hypertension.

1. Radi R. et al.: Biol. Chem. 383:401-409, 2002.
2. Pearce L.L. et al.: Nitric Oxide 5 (2): 128-136, 2001.
3. Cooper C.E. et al.: Biochim. Biophys. Acta 1607: 27-34, 2003.
4. Vaziri N.D. et al.: Hypertension 36: 957-964, 2000.

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EFFECT OF ADAPTATION TO CHRONIC HYPOXIA ON NO SYNTHASES AND APOPTOSIS

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Adaptation of rats to chronic hypoxia is associated with development of hypertrophy and myocardial fibrosis. On the other hand, increased resistance of adapted hearts against all major manifestations of acute ischemia/reperfusion injury was also found. Mechanism of this protection is not fully understood. NO is known to play both, beneficial and detrimental, role in many processes in dependence of its concentration probably due to control of apoptosis. The present study was aimed to investigate the effect of adaptation to intermittent high altitude (IHA) hypoxia on the expression of NO synthases (NOS) and apoptotic regulatory proteins in rat myocardium. Adult male Wistar rats were exposed to IHA hypoxia of 7000 m in a barochamber for 8 h/day, 5 days/week; the total number of exposures was 25. Total contents or activities of regulatory proteins were determined by Western blot analysis or by gelatin zymography. Adaptation to chronic hypoxia enhanced protein levels of cytosolic and particulate eNOS in the RV and moderately increased expression of cytosolic eNOS in the LV was also found. For iNOS in particulate fraction, markedly enhanced expression in the RV and moderately increased in the LV of IHA rats was found. Analysis with antibodies against some pro-apoptotic and anti-apoptotic proteins were also performed. Protein levels of Bax in particulate fraction were moderately decreased in the LV and increased in the RV of adapted hearts. For Bcl-2, moderately enhanced protein expression was indicated in the RV and LV of IHA rats. The levels of inactive and active caspase-3 and cytochrome c were increased in the RV of IHA rat hearts. Western blot analysis displayed increased activation of anti-apoptotic Akt kinase in the LV of IHA rats. For ERK-2 (potential activator of NOS), partial up-regulation in both, cytosolic and particulate, fractions from the RV of adapted rats was found. Zymographic analysis of metalloproteinase 2 (MMP-2) revealed its increased activity in the RV of adapted group. These results show that the adaptation of rat hearts to IHA hypoxia is associated with changes in the expression of NOS and apoptotic regulatory proteins that differ in the two ventricles. The potential consequences of these changes in the cardioprotective mechanism of chronic hypoxia remain to be elucidated.

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ELECTRICAL ACTIVITY OF THE HEART IN THE RATS WITH EXPERIMENTAL HYPERTENSION

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In this study the NO-dependent hypertensive (L-NAME) rats were compared to spontaneously hypertensive rats (SHR). SHR possessed not only higher blood pressure (204±2 vs. 155±2 mmHg), HW/BW and LVW/BW indexes, but more developed myocardial hypertrophy than L-NAME rats as well.

ECG recorded from animals under mild general anesthesia showed smaller amplitude and longer duration of P and T waves as well as QRS complex in SHR than in L-NAME rats. Moreover, both PQ and QT intervals lasted longer in SHR.

Spontaneous frequency of heart isolated from SHR was approximately 30 – 43% slower than the heart frequency recorded from intact animals, and the incidence of ectopic activity was observed. In SHR there were wider data ranges of QRS complex amplitude and QT interval duration compared to L-NAME rats.

Epicardial monophasic action potential (MAP) lasted about 12 % more in SHR than in L-NAME rats allowing increased incidence of late and early after depolarization.

Higher variability in observed ECG parameters together with changes of MAP demonstrate higher susceptibility of hypertrophic myocardium to ectopic electrical activity, especially in SHR than in L-NAME rats.

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SIGNIFICANCE OF ANTIOXIDANTS IN EXPERIMENTAL HYPERTENSION

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Numerous data have documented the positive effect of different antioxidants in cardiovascular diseases including hypertension. NADPH oxidase, xanthine oxidase and uncoupled NO synthase particularly were demonstrated as the main sources of reactive oxygen species (ROS) in spontaneous hypertension. L-NAME-induced hypertension was however, from this point of view, studied much less. Indeed, in L-NAME-induced hypertension developed by inhibiting NO (reactive nitrogen specie) production, the increased ROS might supply decreased NO level.

Nevertheless, in our experimental studies antioxidants such as N-acetylcysteine, melatonin and provinol were able to prevent L-NAME-induced hypertension. Provinol accelerated even regression of this form of hypertension and decreased fibrosis enlargement due to the L-NAME treatment. Increased NO synthase activity and decreased ROS production seemed to be partially responsible for the preventive effect of these antioxidants on L-NAME-induced hypertension. On the other hand, apocynin – NADPH oxidase inhibitor and aspirin – cyclooxygenase inhibitor failed to affect blood pressure in L-NAME-induced hypertension. Neither apocynin nor aspirin affected NO synthase activity modified by L-NAME treatment in the heart and kidney. Both apocynin and aspirin decreased ROS level measured as conjugated diene concentration in the kidney with more significant effect of aspirin. Surprisingly, L-NAME and aspirin cotreatment led to more significant increase of myocardial fibrosis enlargement than L-NAME treatment alone.

It is hypothesized that antioxidants with activating effect on NO synthase and/or stabilizing effect on NO level are able to interfere

successfully with L-NAME-induced hypertension. Decreasing ROS generation without simultaneous improvement of NO synthase activity seems to play rather negative role in this form of hypertension.
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DETERMINATION OF REDOX STATUS DURING DEVELOPMENT AND TREATMENT OF HYPERTENSIO N *IN VIVO*.

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During development of hypertension there are several systems participating in blood pressure increase. The most important seems to be renin-angiotensin-aldosterone system, sympathetic nervous system, metabolic pathway of L-arginin/nitric oxide and oxidative overload. Alterations in these systems might influence development and maintenance of hypertensive disease. Recently it was documented that antihypertensive drugs as ACE inhibitors, AT1 receptor and aldosteron blockers, diuretics and NO donors have significant antioxidant effect, which can be important in hypertension therapy.
The aim of this study is to analyze the methods of oxidant status determination and to define the most suitable methods for determination of redox status *in vivo*. Such determination includes measurement of antioxidant status as well as investigation of processes connected with oxidative stress.
For determination of antioxidant status in plasma, decolorisation assay TEAC-trolox equivalent antioxidant activity is used in a general. In this assay, blue green chromophore $ABTS^+$ is decolorized according to antio xidant level in plasma. Changes in absorbance are detected spectrophotometricaly at 734 nm (1). For determination of ROS/RNI level in different organs of hypertensive rats, fluorescent and chemiluminometric detections are suggested. Nonfluorescent dye 2,7 DCFH- DA was found to be sensitive to peroxynitrite and hydroxyl radicals. After incorporation of dye into cells, it is deacetylised by cell esterases and than oxidised by free radicals to a fluorescent dye with excitation at 488 nm and emission at 520 nm (2). For superoxid detection, chemiluminometry with luminol or lucinogen is one of the most sensitive method (3).
All these methods are useful for evaluation of antioxidant properties of antihypertensive drugs *in vivo* as well as for evaluation of changes in oxidative status during development and treatment of hypertension.
1.Free Radical Biology and Medicine 26, Nos 9/10, pp.1231-1237, 1999
2. Biochemical Pharmacology 65, pp 1575-1582, 2003
3. Free Radical Biology and Medicine 38, pp 262-270, 2005

INFLUENCE OF RED WINE POLYPHENOLS ON CHANGES IN COLLAGEN TYPE I. AND III. PRESENCE IN THE VASCULAR WALL AFTER DAMAGE INDUCED BY CCl_4

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Introduction: Our previous studies demonstrated that tetrachlormethane (CCl_4) leads to damage not only of parenchymatous organs but also to a serious deterioration of vascular endothelium. This study analyses changes in the vascular wall composition after chronic CCl_4 application. Preventive effect of polyphenols contained in red wine on develo pment of cardiovascular diseases has been described in numerous studies of the human population and on experimental animal models.
Aim: The effect of polyphenols isolated from red wine on the presence of collagens in the vascular wall of experimental anim als administered CCl_4 was evaluated.
Methods: Male Wistar rats were administered 0.75 ml CCl_4 /kg body weight subcutaneously two times a week for 12 weeks. Polyphenols were given parallely with CCl_4 or during the 3-week regression phase of the experiment. Paraffin slices 5 µm thick were stained with sirius red and evaluated in a fluorescence microscope. The presence of collagen type I. and III. was morphometrically and statistically evaluated.
Results: Chronic administration of CCl_4 for 12 weeks lead to accumulation of collagen type I. and a decrease of type III. collagen when compared with control animals. Oral administration of polyphenols from red wine in the dose of 30mg/kg /day in drinking water, parallely or in the regression phase, significantly corrected the changes introduced by chronic CCl_4 intoxication. Polyphenols alone increased the presence of type III. collagen in the vascular wall.
Conclusion: Our results demonstrated a significant protective effect of red wine polyphenols from the increase of the unfavorable collagen type I. accumulation in the vascular wall. This positive effect was confirmed also by an increase of the favorable type III. collagen that increases elasticity and stability of the vascular wall.
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PROVINOL AND ALLERGEN – INDUCED HYPERREACTIVITY OF THE TRACHEAL SMOOTH MUSCLE

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Nitric oxide (NO), as a basic mediator of inhibitory nonadrenergic and noncholinergic (i-NANC) neurotransmission in the airways, plays important role in the pathophysiology of the respiratory diseases. Deficiency of NO is probably one of the important factors in the allergen – induced hyperreactivity of the airways.
Using a guinea pig model of ovalbumin (OVA) induced airway inflammation, the aim of the study was to determine the acute and long-lasting effect of Provinol (red wine polyphenolic compounds) on the tracheal smooth muscle reactivity by in vitro method. Second phase of the study was to evaluate the role of NO in the bronchodilatory effect of Provinol.
Amplitude of the tracheal smooth muscle contraction to bronchoconstrictor mediators - histamine (10^{-8} –10^{-3} mol.l^{-1}), acetylcholine (10^{-8} –10^{-3} mol.l^{-1}) and to allergen (OVA 10^{-5}-10^{-3}g/ml), was used as a parameter of tracheal smooth muscle reactivity. In acute experiments the isolated tracheal strips were in organ bath pre-treated 30 min with Provinol (0.01 mg/ml), and 30 min with Provinol in combination with L-NAME (10^{-6} mol.l^{-1}). In chronic experiment experimental animals were treated 14 days with Provinol (20mg/kg/day) and Provinol in combination with L-NAME (40 mg/kg/day).
Provinol antagonised OVA-induced contraction of the tracheal smooth muscle strips prepared from guinea pigs after 2 weeks of OVA-sensitization, and this reaction was partially inhibited with L-NAME. 30 minutes incubation of the tracheal smooth muscle with Provinol resulted in a decreased amplitude of contraction to bronchoconstrictor mediators – histamine and acetylcholine and the effect of Provinol was partially diminished by L-NAME (mainly in low doses of bronchoconstrictor). Similar picture of changes was observed after chronic 14 days administration of Provinol. In conclusion, Provinol inhibited the allergen and spasmogen induced contraction of the tracheal smooth muscle in OVA-sensitized guinea pigs, and this mechanism of action is probably partially mediated through the metabolism of NO.

EFFECT OF ROOIBOS TEA ON LIVER DAMAGE INDUCED BY EXPERIMENTAL TOXIC INJURY

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Rooibos tea containing wide range of polyphenolic compounds has been documented to exert potent antioxidant activity. This study evaluates the effect of Rooibos (*Aspalathus linearis*) tea on liver damage induced by chronic carbon tetrachloride (CCl_4) experimental intoxication. Male Wistar rats were divided into 3 groups: a group receiving CCl_4 for 6 weeks, a group with free access to Rooibos tea instead of water supply in the recovery period after 6 weeks of toxicity induction (regression group) and a group that received Rooibos tea during the toxicity induction (preventive group). The animals were killed after 0, 21 and 42 days following the toxicity induction period. Groups that were not treated by CCl_4 with and without Rooibos tea administration were regarded as control groups. CCl_4 administration led to liver fibrosis and steatosis and an increase of laboratory parameters typical for liver damage. Parallel tea administration in the preventive group significantly reduced the fibrosis with a complementary steatosis increase. The decrease of free radicals formation was documented by malondialdehyde evaluation in the liver tissue. No differences of the decreasing fibrosis levels between the groups with and without Rooibos tea treatment after 21 and 42 days of recovery was observed. The increased steatosis level remained also after 21 days of recovery and was significantly higher when Rooibos tea was administrated. The fibrosis level did not decrease significantly during the regression period in the groups without the tea administration. NO synthase activity in the liver was increases by Rooibos administration. It is likely that Rooibos tea compounds can participate on reduction of the liver damage when given during the intoxication period. The antioxidant activity stimulation of NO synthase activity can explain these effects. One has to recognize that the free radicals may play a not negligible role in the organism as an important factor of tissue recovery after toxic injury. This can explain the extended healing period when Rooibos tea was administrated during the recovery period. Other factors, as well as the possible pro-oxidant effect of NO produced in high amount when Rooibos tea is administrated, cannot be excluded.

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LIPID PEROXIDATION INDUCED BY HYPOBARIC HYPOXIA CONDITIONS IN RAT BRAIN AND THE POSSIBLE PREVENTION BY DIFFERENT PRETREATMENTS

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There is growing evidence that reactive oxygen (ROS) and nitrogen species and increased lipid peroxidation play important role in the initiation and progression of many human dysfunctions associated with diseases such as diabetes mellitus, atherosclerosis, ischemic heart disease, hypertension, stroke, neurodegenerative diseases etc. Lipid peroxidation influences numerous cellular functions through membrane-bound enzymes, transport processes or metabolic activities. In our experiments we used as model Wistar rats exposed to hypobaric hypoxia. We observed that the acute exposure to hypobaric hypoxia increased thiobarbituric acid-reactive substances (TBARS; a reliable index of lipid peroxidation) in the rat brain but this response was dependent on the age of animals. Generally, we observed smaller TBARS increase in female than in male brain. During the ontogeny of central nervous system the lowest resistance to hypoxia was found in 21-day-old-rats. As concerns erythrocyte ion transport, we did not observe any significant changes of Na^+ content or activity of Na^+-K^+ pump, Na^+-K^+ cotransport and cation leaks. In the case of metabolic activities we detected the increased levels of lactate and pyruvate in brain and blood after the exposure to hypobaric hypoxia. The activity of the key enzyme of anaerobic glucose metabolism, lactate dehydrogenase (LDH) increased in the brain with the age of rats, but it did not change in blood serum. The exposure to hypobaric hypoxia did not change LDH activity in the brain. The aim of our study is to determine how the different pretreatments (administration of α-tocopherol, L-carnitine or phosphocreatine before the hypobaric hypoxia exposure) could affect lipid peroxidation and glucose metabolism. All above mentioned pretreatments decreased brain TBARS levels in comparison with hypobaric hypoxic rats. L-carnitine and phosphocreatine pretreatments decreased also pyruvate and lactate levels. However, we did not find any significant differences in brain LDH activity after L-carnitine pretreatment. We conclude that L-carnitine could probably change the kinetic of oxidative decarboxylation of pyruvate due to the increased accessibility of acetyl-CoA from lipid metabolism influenced by L-carnitine.

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LATE EFFECTS OF EARLY INTERVENTION: TRANSIENT CAPTOPRIL TREATMENT OF SHR IN JUVENILE CRITICAL PERIOD FOR HYPERTENSION

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Since the roots of many important diseases can be found in the early stages of development, the attention should be focused to the effects of early antihypertensive interventions on further development of genetic hypertension. It was repeatedly reported that transient treatment of prepubertal SHR with ACE inhibitors prevents hypertension development and attenuates BP recovery after drug withdrawal. The aim of our study was find mechanism responsible for captopril-induced prevention of spontaneous hypertension and the cause of long-term BP attenuation of hypertension development after early captopril treatment. Young (4-week-old) SHR were treated with captopril (100 mg/kg/day) for 6 weeks. Basal BP, BP response to i.v. nifedipine, BP changes after consecutive blockade of RAS (losartan), SNS (pentolinium) and NOS (L-NAME) as well as residual BP after nitroprusside injection were measured in conscious rats at the end of active treatment and after 4 or 20 weeks of drug withdrawal. SHR were characterized by enhanced sympathetic vasoconstriction, augmented nifedipine-induced BP fall, greater vasodilator deficit and increased residual BP. Captopril treatment of young SHR prevented all above alterations except of vasodilator deficit. Low residual BP and normalized BP response to nifedipine persisted after captopril withdrawal and could be detected during long-term attenuation of hypertension development, i.e.20 weeks after drug withdrawal. It can be concluded that the blockade of central angiotensin effects decreased enhanced sympathetic tone in young SHR, resulting thus in reduction of Ca^{2+} influx during tonic vascular contraction and attenuation of hypertrophic vascular remodeling.

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THE ROLE OF CHRONIC CAPTOPRIL (CPT) AND N-ACETYL-CYSTEINE (NAC) TREATMENT IN L-NAME HYPERTENSION IN THE RAT

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The long-term control of BP is known to be based upon the balance of vasoactive systems. Our recent studies revealed that experimental hypertension in the rat elicited by chronic L-NAME treatment resulted in increased sympathetic vasoconstriction and attenuated NO-dependent vasodilation. In our study we tried to reveal the role of chronic captopril or NAC treatment in their antihypertensive action. Group of L-NAME treated (60 mg/kg/day for 5 weeks) adult Wistar males was compared to groups of animals in which L-NAME (LN) was combined with simultaneous administration of captopril (LN+CPT, 100 mg/kg/day) or N-acetylcysteine (LN+NAC, 1,5 g/kg/day). In conscious cannulated rats the basal BP as well as its acute responses to consecutive i.v. administration of losartan (10 mg/kg), pentolinium (5 mg/kg), L-NAME (30 mg/kg) and sodium nitroprusside (NP, 20 μg/kg) were determined.

Chronic CPT treatment almost prevented the development of L-NAME hypertension, whereas only moderate BP reduction was observed after NAC treatment (by 16 mmHg, -10 %). Major effects of chronic captopril treatment was caused by significant reduction in both sympathetic BP component (by 18 mmHg, -23 %) and residual BP measured at full NP-induced vasodilation (6 mmHg, -13 %). Chronic NAC treatment significantly attenuated vasodilator deficit (9 mmHg, -35 %) due to enhanced NO-dependent vasodilation (13 mmHg, +59%). We conclude that decrease of central sympathetic activity as well as reduction of structural vascular resistance caused by chronic captopril treatment prevents L-NAME hypertension. Attenuated L-NAME hypertension via augmentation of NO-dependent vasodilation was observed after chronic administration of NAC.

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BRAIN NITRIC OXIDE SYNTHASE EXPRESSION IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR) DURING THE CHRONIC CAPTOPRIL TREATMENT

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Neuronal nitric oxide synthase (nNOS) is a one of three isoforms of NOS which are involved in cardiovascular homeostasis. A centrally generated nitric oxide participates in reduction of sympathetic activity in contrast to central angiotensin II which contributes to enhanced sympathetic tone. We suppose that elevated blood pressure of SHR is mainly caused by high activity of sympathetic nervous system (SNS) which is driven by abnormal central nervous mechanisms. Quadri et al. (J Hypertens 2003; 21:1687) reported altered activity of nNOS in hypothalamus and brainstem of SHR. The aim of our study was to determine the expression level of nNOS protein in different brain regions (brainstem and cerebellum) by Western blot analysis in SHR (mean arterial pressure 136±13 mm Hg), SHR and Wistar-Kyoto rats (WKY) treated for 6 weeks with captopril (100 mg/kg/day, 101±11 mm Hg, 85±4 mm Hg, respectively) and compared with untreated normotensive WKY (106±5 mm Hg). These blood pressure changes were mainly caused by alterations in sympathetic vasoconstriction. Irrespective of brain region investigated Western blot analysis indicated similar level of nNOS expression as in WKY: SHR (brainstem: 93±6 %; cerebellum: 103±5 % of WKY values), and WKY treated with captopril (brainstem: 90±7 %; cerebellum: 94±11 % of WKY values). Moreover, chronic captopril treatment of SHR which completely normalized their blood pressure did not influence nNOS expression in either brain region (93±8 and 104±8 % of WKY, respectively). Our results clearly show that altered regulation of sympathetic tone and resulting high blood pressure in SHR is connected with the injurious influence of central angiotensin II rather than with the defect in nitric oxide central generation.

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DIFFERENT EFFECTS OF INDAPAMIDE AND HYDROCHLORO-THIAZIDE TREATMENT ON THE SPONTANEOUS AND L-NAME INDUCED HYPERTENSION.

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This study was aimed to compare the preventive effect of thiazide-like diuretics hydrochlorothiazide (HCT) and indapamide on blood pressure development in spontaneous and L-NAME induced hypertension. Young 6-week-old male SHR were treated with HCT (10 mg/kg/day) or indapamide (1 mg/kg/day) for six weeks. Adult male Wistar Kyoto rats were treated with L-NAME (40 mg/kg/day) alone, or L-NAME + HCT, or L-NAME + indapamide for seven weeks. Nitric oxide synthase (NOS) activity was determined in the left ventricle, aorta and kidney, eNOS protein expression was determined in the left ventricle and conjugated dienes (CD) were detected in the kidney. Both drugs partially attenuated systolic blood pressure rise in young SHR (control: 171±2, HCT: 154±4, indapamide: 157±3 mmHg). Indapamide, in contrast to HCT, significantly increased NOS activity in the aorta without modulating eNOS protein expression in this tissue. Both indapamide and HCT failed to modify NOS activity and/or eNOS protein expression in the heart and kidney. Indapamide attenuated concentration of reactive oxygen species (ROS) measured as decreased concentration of CD in the kidney. HCT treatment did not show antioxidant effect in any tissue investigated. 7-week L-NAME treatment increased blood pressure, elevated NOS activity in the aorta, heart and kidney and decreased CD concentration in the kidney. In comparison to the L-NAME treatment alone, indapamide preserved NO synthase activity on the control level, while HCT did not affect NO synthase activity modified by L-NAME treatment. Chronic L-NAME and indapamide or HCT treatment led to the moderate enhancement of endothelial NO synthase protein expression in the left ventricle. Our study demonstrated that HCT and indapamide (even if used in ten times lower dose than HCT) decreased blood pressure comparably. HCT did not affect NO synthase activity and CD concentration in both spontaneously and L-NAME treated rats. In contrast, indapamide modulate NO synthase activity in the models of experimental hypertension investigated. Thus the antihypertensive effect of indapamide involves not only its diuretic effect, but also modification of the imbalance between NO and ROS production.

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EFFECT OF CHRONIC PROVINOL TREATNENT ON THE DEVELOPMENT OF L-NAME INDUCED HYPERTENSION.

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Red wine polyphenols have been reported to possess beneficial properties for preventing cardiovascular diseases but their effects during chronic L-NAME treatment have not been elucitated. The effect of the red wine polyphenols, Provinol, on activity of NO synthase (NOS), conjugated dienes (CD) concentration, arterial hypertension as well as left ventricular hypertrophy and femoral artery relaxations were investigated in rats during 4- and 7- week- L-NAME treatment. Rats were divided into six groups: controls, groups treated with N^G-nitro-L-arginine methyl ester (L-NAME, 40 mg/kg/day) and groups receiving Provinol (40 mg/kg/day) plus L-NAME for 4 or 7 weeks. 4-week-L-NAME treatment led to decrease NO synthase activity and increased concentration of CD in the heart and aorta as well as to hypertension development. However, prolonging L-NAME effect to 7 weeks increased NO synthase activity in both organs and decreased CD concentration in the kidney without any additional changes of blood pressure. In comparison to the L-NAME treatment alone, Provinol increased NO synthase activity and decreased CD concentration during 4-week-cotreatment with L-NAME, however, it failed to affect these parameters during 7-week-cotreatment with L-NAME. Moreover, Provinol markedly reduced the increase of blood pressure caused by chronic L-NAME treatment. Chronic co-treatment L-NAME and Provinol led to the moderate enhancement of endothelial NOS protein expression in the heart and aorta. In addition, Provinol corrected the augmented constriction and attenuated endothelium-dependent relaxation of femoral artery in L-NAME treated rats. Our results provide evidence that the increase of NOS activity and prevention of oxidative stress should be responsible for antihypertensive Provinol

effect during 4-week-co-treatment with L-NAME. Since L-NAME itself, increased NO synthase activity and decreased level of reactive oxygen species in the heart and aorta during 7-week-treatment, without reduction of blood pressure, other particular regulatory systems may be also responsible for antihypertensive effect of Provinol during 7-week-co-treatment. Thus, Provinol partially prevents L-NAME induced hypertension and vascular dysfunction via the different mechanisms depending on the duration of L-NAME and Provinol treatment. Supported by VEGA 2/6148/26, 1/3429/06 and APVT 51-027404.

NITRIC OXIDE SYNTHASE ACTIVITY AND BLOOD PRESSURE IN THE RATS WITH VARIOUS FAMILY HISTORY OF HYPERTENSION

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The aim of this study was to determine the basal values of nitric oxide (NO) synthase activity in selected parts of the cardiovascular and neuroendocrine system in rats with various family history of hypertension and to investigate the relations between basal NO synthase activity and blood pressure. Wistar rats (W) were used as the model of normotensive rats, without the history of hypertension. Spontaneously hypertensive rats (SHR) were used as the model of rats with two hypertensive parents. Borderline hypertensive rats (BHR, offspring of one Wistar and one SHR rat) were used as the model of rats with one hypertensive parent. We used a group of BHR with normotensive Wistar mother and SHR father (wBHR) as well as a group of rats with SHR mother and Wistar father (sBHR). All rats were kept in standard conditions, four rats per cage, 480 cm^2 per rat, until the age of 20 weeks, with water and food at libitum. Blood pressure (BP) was determined at the age of 12, 13, 15, 18 and 20 weeks using tail-cuff plethysmography. NO synthase activity was determined by conversion of [^{3}H]arginine to[^{3}H]citrulline in the aorta, left ventricle, right ventricle, hypothalamus, pituitary and adrenal glands of all rats. BP of W, wBHR, sBHR and SHR rats at the end of experiment was 111±3, 129±2, 132±2 and 185±2 mm Hg, respectively. NO synthase activity in the aorta of W rats was 4.7±0.5 pmol/min/mg and it was increased significantly in wBHR, sBHR and SHR rats ($p<0.02$ vs.W, in all groups). In the left and right ventricle, NO synthase activity was significantly higher in SHR ($p<0.01$ vs. W). In hypothalamus and pituitary, the lowest values of NO synthase activity were observed in wBHR ($p<0.01$ vs. W) without significant differences among W, sBHR and SHR. In adrenal glands, the lowest values were observed in W and significantly higher values were found in both wBHR and sBHR ($p<0.04$ vs. W). Data suggest that basal NO synthase activity was elevated in the aorta of all rats with family history of hypertension (wBHR, sBHR, SHR) as well as in the heart of rats with two hypertensive parents. The elevated basal NO synthesis in vascular system may represent an adaptive mechanism to elevated sympathetic activation of rats with at least one hypertensive parent. On the other hand, no relations between BP and basal NO synthase activity in the hypothalamus, pituitary or adrenal glands were observed.
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DIFFERENT EFFECT OF CROWDING ON NITRIC OXIDE SYNTHASE ACTIVITY IN CARDIOVASCULAR SYSTEM IN OFFSPRING OF WISTAR AND SHR MOTHERS

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The aim of this study was to determine the effect of social stress produced by crowding on nitric oxide (NO) synthase activity in the heart and aorta in rats with normotensive Wistar (W) or spontaneously hypertensive (SHR) mothers. Wistar-mothered rats were offspring of either Wistar (W group) or SHR (wBHR group) fathers. SHR-mothered rats were offspring of SHR (SHR group) or Wistar (sBHR group) father. Twelve weeks old males of all phenotypes were randomly divided into control (four rats per cage, 480 cm^2 per rat) or stressed (five rats per cage, 200 cm^2 per rat) group with water and food at libitum. Blood pressure (BP) was determined before crowding and on the 1st, 3rd, 6th and 8th week of experiment using tail-cuff plethysmography. NO synthase activity was determined by conversion of [^{3}H]arginine to [^{3}H]citrulline in the aorta and left ventricle. BP of control W, wBHR, sBHR and SHR rats at the end of experiment was approximately 111±3 129±2, 132±2 and 185±2mm Hg, respectively. Eight weeks of crowding increased BP significantly only in sBHR and SHR rats ($p<0.02$ vs. control). Crowding significantly reduced NO synthase activity in the aorta and left ventricle of SHR rats and partial reduction was observed also in the sBHR group. No changes in NO synthase activity in the aorta and left ventricle were observed in W and wBHR rats. The analysis of NO synthase activity revealed a significant reduction of NO production in the aorta and left ventricle of crowding-exposed SHR-mothered rats (SHR plus sBHR) vs. control. This effect was not observed in Wistar-mothered (W plus wBHR) rats. In conclusion, social stress produced by crowding reduced NO synthesis in the aorta and left ventricle of SHR-mothered rats while no changes in NO production were observed in offspring of normotensive mothers. Reduced NO production in the aorta and left ventricle of SHR-mothered rats was associated with significant elevation of BP. This suggests that male descendants of hypertensive mothers were more susceptible to crowding than descendants of normotensive mothers and that reduced NO production in their cardiovascular system may result in development of hypertension. On the other hand, crowding supposedly did not represent a significant risk factor for development of hypertension in offspring of normotensive mother. Supported by the grants Nos. APVT-51-018004 and VEGA 2/4156/25.

MESENTERIC ARTERY REACTIVITY OF RATS WITH DIFFERENT PREDISPOSITION TO HYPERTENSION - EFFECT OF CROWDING STRESS

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The influence of stress on bioavailability of nitric oxide (NO) in the cardiovascular system is not known so far. Changes in NO bioavailability, both positive or negative, may result in alteration of blood vessel function and consequently in blood pressure changes. The aim of this work was to investigate the effect of social stress due to high population density – crowding stress - on endothelium-dependent relaxation of the rat superior mesenteric artery (SMA) in three various rat strains with different genetic predisposition to hypertension.
Experiments were performed on normotensive rats (Wistar), spontaneous hypertensive rats (SHR) and borderline hypertensive rats (offspring of SHR dams and Wistar sires, sBHR). The rats were exposed to 8-week crowding stress (5 rats/cage 25/40/15 cm, cca 200 cm^2/rat). Control rats were 4 per cage (35/55/20 cm, cca 480 cm^2/rat). Blood pressure was measured using tail-cuff plethysmography. Endothelium-dependent relaxation of SMA rings was studied *in vitro* under isometric conditions. We evaluated the responses of phenylephrine-precontracted preparations (1µmol/l) to acetylcholine (0.01 – 10µmol/l) before and after inhibition of NO synthase (100 µmol/l N^G-nitro-L-arginine methyl ester - L-NAME) and prostaglandin synthesis with indomethacin (10 µmol/l).
In control conditions, SHR and sBHR rats had significantly higher blood pressure compared to Wistar rats (185±2, 132±2 and 111±1 mm Hg, respectively). Eight weeks of crowding induced the increase of blood pressure of SHR and sBHR rats (193±2 and 145±3 mm Hg, $p<0.05$ vs. control), but not of Wistar rats (112±2 mm Hg). In functional

studies on SMA rings, the smallest L-NAME-resistant portion of endothelium-dependent relaxation was found in SHR rats. Responses of SMA to acetylcholine were depressed in both the sBHR and SHR group, while Wistar rats responded to stress with depression only in L-NAME-resistant vasodilatation.

The results showed the smallest endothelial relaxation with the smallest L-NAME-resistant portion in animals with the highest blood pressure, i.e. in SHR rats. Changes in endothelium-dependent relaxation evoked by crowding stress seem to depend predominantly on changes in the NO-portion of endothelial relaxation. Supported by VEGA grants Nos. 2/5009/25, 2/5129/25, 2/4156/25 and APVT-51-018004.

EFFECT OF CROWDING STRESS ON BLOOD PRESSURE AND NEUROGENIC CONTRACTIONS OF ISOLATED VESSELS IN BORDERLINE HYPERTENSIVE RATS.

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Borderline hypertensive rats are very susceptible to various forms of stress but not much is known about the stimuli which contribute to the deterioration and stabilization of hypertension. We investigated the effect of 8-week lasting crowding stress on systolic blood pressure (SBP) and contractile responses of isolated blood vessels to adrenergic stimuli in two models of borderline hypertension. Experiments were carried out on three groups of animals: a) normotensive Wistar rats; b) borderline hypertensive rats (offspring of SHR dams and Wistar sires, sBHR); c) borderline hypertensive rats (offspring of Wistar dams and SHR father, wBHR). Half of rats was exposed to 8-week crowding stress (5 rats/cage 25/40/15cm, cca 200 cm^2/rat). Control normotensive rats were 4 per cage (35/55/20 cm, cca 480 cm^2/rat). Systolic blood pressure was measured by tail-cuff plethysmography at the end of the study. Rings of isolated mesenteric artery and longitudinal segment of portal vein were mounted in organ baths for measurement of isometric contractile force. Neurogenic contractions of mesenteric artery and portal vein were elicited by electrical stimulation of perivascular nerves. The trains of 0.5ms rectangular pulses of supramaximal intensity (>30V) at frequencies 1-32 Hz for a period of 20s were used. Systolic blood pressure of non-stressed animals in Wistar, sBHR and wBHR rats at the end of experiment was 111±2, 132±1 and 129±1 mmHg, respectively. Long-lasting crowding stress increased SBP in sBHR to 145 mmHg ($P<0.05$), but it was not significantly changed in other two groups of rats. Electrical field stimulation caused frequency-dependent contractions of mesenteric artery and portal vein in Wistar, sBHR and wBHR. Contractions of mesenteric artery in sBHR were enhanced in comparison with those in Wistar rats and wBHR. In stressed animals the pattern and magnitude of neurogenic contractions were similar and were not significantly different among investigated groups of animals. In the stress condition the dose-response curve to noradrenaline in sBHR was shifted to the left but it was not significantly changed in normotensive Wistar rats and wBHR. The results demonstrate that long-lasting crowding stress increased SBP and arterial sensitivity to noradrenaline in sBHR. VEGA 2/3166/25, 2/4156/25 and APVT-51-018004.

SUBCELLULAR CHANGES IN THE HEART AND AORTA OF BORDERLINE HYPERTENSIVE RATS (BHR) IN RESPONSE TO SOCIAL STRESS

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Hypertension is known to be associated with a high risk of heart failure accompanied with vascular pathology. In subjects with genetic predisposition to hypertension, stress can represent an important trigger in etiology of heart disease. The aim of our study was to examine the effect of social stress due to crowding on ultrastructure of myocardium and aorta of rats with family history of hypertension. Male adult borderline hypertensive rats (BHR, offspring of Wistar dam and spontaneously hypertensive sir) were exposed to 6-week crowding stress (5 rats/cage, 200cm^2/rat). Control rats were kept 4 rats/cage (480 cm^2/rat). Blood pressure was determined non-invasively on the tail. Basal blood pressure of BHR was 132.2 mm Hg. Crowding stress increased significantly blood pressure ($p<0,01$ vs. basal value) and the relative weight of the right ventricle, without the alterations in the relative weight of the left ventricle. Crowding stress had no influence on NO synthase activity in the heart, however enzyme activity in the aorta was reduced significantly by 52% ($p<0,005$ vs. control). The heart and aorta were perfusion fixed with 2% glutaraldehyde and small tissue blocks of left and right ventricle and aortic rings were routinely processed for electron microscopy. The immunofluorescence of endothelial inflammatory von Willibrand factor (vWF) in aorta and heart and connexin-43 in intercellular connections „gap junctions“ of heart was determined using polyclonal antibody rabbit anti-human vWF and monoclonal antibody mouse anti-connexin43. Ultramicroscopy showed that experimental stress in BHR male rats induced moderate subcellular alterations in cardiomyocytes of both ventricles. They were characterized with swollen mitochondria, frequent intracellular vacuoles, and extension of T-system. Some cardiomyocytes in the right ventricle revealed structural markers of hypertrophy. Most capillaries formed regular lumen and endothelial cells had normal structure. However, capillaries located nearby hypertrophied cardiomyocytes revealed structural markers of angiogenesis. On the other hand, some endothelial cells were abnormal suggesting endothelial dysfunction. In aorta, stress resulted in serious injury of endothelial cells: they were edematous, contained enhanced amount of vacuoles, lysosomes and Weibel-Palade bodies indicating endothelial dysfunction and activation of inflammation processes. Most smooth muscle cells were characterized with normal architecture, but some of cells contained vacuoles with degraded membrane structures. Endothelial vWF immunoreaction was more intensive in stressed rats comparing to controls. While, fluorescence signal for connexin-43 was locally decreased in stressed rats. Results indicate that social stress induced serious subcellular alterations of aortic endothelial cells indicating their high susceptibility on stress, while moderate structural alterations of the cardiomyocytes and capillaries that suggests injury as well as activation of adaptation processes in cardiovascular system of borderline hypertensive rats.

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GENDER DIFFERENCES IN OPEN FIELD BEHAVIOUR AND BLOOD PRESSURE OF RATS WITH VARIOUS FAMILY HISTORY OF HYPERTENSION EXPOSED TO CHRONIC SOCIAL STRESS

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This study investigated the gender differences in the open field behaviour and blood pressure (BP) in normotensive and borderline hypertensive rats (BHR) in response to chronic social stress. Adult males and females Wistar and BHR (offspring of Wistar dams and spontaneously hypertensive sires) were exposed to crowding stress (200 cm^2/rat, 5 rats/cage) for 6 weeks. Four rats/cage (480 cm^2/rat) were kept in the control groups of animals. BP and activity in the open field were determined before experiment and after 1, 3 and 6 weeks of the stress exposure. Basal BP of BHR was higher than in Wistar rats ($p<0.001$) in both males and females. Horizontal and vertical activity of BHR males and females was elevated compared to Wistar rats ($p<0.01$). Females of both phenotypes were more active than males ($p<0.01$). Crowding stress resulted in a slowed-down between-session habituation, a significant

elevation of BP and an increase of relative adrenal gland mass in BHR males. These alterations were not observed in normotensive males. In crowded females, stress failed to affect BP and there was no observed between-session habituation in their horizontal and vertical activity compared to controls.

The results indicate that behavior of rats in the open-field was dependent on their gender and blood pressure. Females of both phenotypes were more active than males and they were behaviourally more susceptible to crowding stress. However, altered open field behavior of crowded females was not associated with changes in BP. On the other hand, crowding resulted in slowed-down habituation associated with the increase of BP and in BHR males. Thus, chronic social stress produced by crowding seems to represent significant risk factor for development of stress induced hypertension in males with genetic predisposition to high blood pressure, while females appeared to be more sensitive to behavioral alterations.

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REGRESSION OF L-NAME-INDUCED HYPERTENSION: THE ROLE OF NO-PATHWAY

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N^G-nitro-L-arginine-methyl ester (L-NAME)-induced hypertension is an attractive model of experimental hypertension. However the mechanisms contributing to its regression are not completely understood. We aimed to investigate, whether the regression of L-NAME hypertension is associated with improved NO-synthase (NOS) pathway. Four groups of Wistar rats (n=8 each) were investigated: 5-week control (5-Ctr), L-NAME (5 weeks 40 mg/kg/day), spontaneous recovery (SR, 5 weeks L-NAME followed by 3-week recovery) and 8-week control (8-Ctr). Blood pressure (BP) was measured invasively. NOS activity in the aorta was determined by measuring the conversion of radioactive L-arginine to L-citrulline. Using Mulvany myograph, the normalized inner diameter of the femoral artery, the relaxation to acetylcholine (ACh) of nor-epinephrine (NE)-precontracted femoral and small mesenteric arteries and the influence of L-NAME-preincubation on NE-contraction were evaluated. L-NAME caused BP enhancement, decreased NOS activity, decreased inner arterial diameter, impaired ACh-relaxation and decreased sensitivity to L-NAME-preincubation. By SR complete restoration of NOS activity took place. However, the BP was reduced only by 10% with proportional changes in the ACh-relaxation and sensitivity to L-NAME-preincubation. The inner arterial diameter remained unaffected. We conclude that L-NAME administration leads to alterations in vessel reactivity which may maintain enhanced blood pressure even after restoration of NOS activity.

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RED WINE POLYPHENOLS CORRECT ALTERED VASCULAR FUNCTION AND STRUCTURE IN RAT THORACIC AORTA

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Many experimental studies have demonstrated the beneficial effects of antioxidant treatment on CCl_4 induced tissue injury. Red wine polyphenolic compounds have been shown to have antioxidant, vasorelaxant, antihypertensive and other protective properties on cardiovascular system. The aim of this study was to investigate their effect on the vascular reactivity and morphology of rat thoracic aorta (TA) affected by CCl_4 chronic treatment. Male Wistar rats were divided into 6 groups: control group, group receiving 12 weeks CCl_4 subcutaneously (0,5 ml/kg) two times a week, group that after that was allowed to recover for a 3-week regression period, the same groups that received in addition red wine extract with polyphenols in drinking water (30 mg/kg/day) and a group receiving only polyphenols for 12 weeks. NO-synthase (NOS) activity was determined in the left ventricle. Functional ability of vessel wall was determined by studying of noradrenaline-induced (NA, 10^{-9}-10^{-6} mol/l) contractile and acetylcholine-induced (ACh, 10^{-8}-10^{-5} mol/l) relaxant responses on isolated rings of TA. Wall thickness (WT), cross-sectional area (CSA) and inner diameter (ID) of TA were measured in light microscopy. NOS activity in the heart was unchanged after CCl_4 - treatment, but it was significantly increased in all groups treated by polyphenols. Long-term CCl_4-administration resulted in the significant inhibition of ACh-induced relaxation of TA which was accompanied by endothelial injury and dystrophy. Simultaneous administration of CCl_4 with polyphenols as well as polyphenols-treatment during 3 weeks of regression showed endothelium protective effect and refreshed ACh-induced relaxation. Besides inhibited relaxation, chronic CCl_4-treatment reduced NA-induced contraction of TA. Administration of polyphenols during regression period increased and normalized the inhibited contraction to adrenergic stimuli. Long-term polyphenol-treatment significantly increased the WT/ID index compared to group treated by CCl_4. Similarly, the CSA of TA in the regression group was significantly augmented after chronic administration of polyphenols. Results suggest that red wine polyphenols correct vascular reactivity of rat thoracic aorta altered by CCl_4 -induced injury. Polyphenols reveal endothelium-protective effect and positive influence on reparative processes in the vascular wall. Supported by VEGA N° 1/1171/04 and APVT:51-017902.

EFFECT OF MELATONIN ON ACTIVITY OF SMOOTH MUSCLE OF RABBIT CONDUIT ARTERIES

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Melatonin, the main pineal gland hormone, is known as a biological mediator of circadian rhythms, sleep, sexual behaviour, immunologic and cardiovascular functions. Role of melatonin in regulation of vascular tone remains controversial (1). High concentrations of melatonin have been shown to relax isolated rat pulmonary artery and its extralobular branches (2). In present study we have examined the effect of melatonin on rabbit pulmonary arteries and extended the investigation also to other rabbit arteries (thoracic aorta, mesenteric artery). Isolated arteries were cut into rings and mounted in tissue baths for measurement of isometric contractile force. Neurogenic contractions were elicited by electrical stimulation of perivascular adrenergic nerves by means of two platinum plate electrodes positioned on either side of the arterial ring. The trains of 0,5 ms rectangular pulses of supramaximal intensity (>30V) at frequencies 1 – 4 Hz for a period of 20 s were used. Melatonin (10^{-6} - 3.10^{-5} mol/l) itself caused neither contraction nor relaxation of isolated rabbit thoracic aorta. After precontraction with phenylephrine (10^{-6} mol/l) melatonin in low concentration did not change the vascular tone, in high concentration melatonin (3.10^{-5} mol/l) relaxed precontracted aorta (20,1 ± 6,6%, $P < 0.01$). Melatonin (3.10^{-5} mol/l) induced also relaxation of pulmonary artery (42,3 ± 6,7%, $P < 0.001$) and main pulmonary artery (15,4 ± 3,6%, $P < 0.01$). The application of NO synthase inhibitor N^7-nitro-L-arginine methylester (L-NAME, 10^{-6} mol/l) prior to phenylephrine did not influence the magnitude of melatonin induced maximum relaxation of pulmonary arteries. High concentration of melatonin (10^{-5} mol/l)

inhibited neurogenic contractions of pulmonary (24,7 ± 4,4%, $P < 0.001$) and mesenteric arteries (15,9 ± 5,8%, $P < 0.05$). Melatonin (10^{-5} mol/l) did not change endothelial dependent relaxation of rabbit aorta induced by acetylcholine ($10^{-9} – 10^{-5}$ mol/l). These experiments showed that melatonin relaxed phenylephrine precontracted blood vessels and inhibited neurogenic contractions of all tested rabbit arteries. Failure of melatonin to change endothelium-dependent relaxation in aorta indicates that this drug does not influence the NO production in large arteries.

(1) Masana MI et al.: J. Pharmacol. Exp. Ther. 302: 1295-1302, 2002
(2) Líšková S and Török J: Physiol. Res. 52: 51P, 2003

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COMPARISON OF FEMORAL ARTERY RELAXATION AFTER ACUTE AND CHRONIC ADMINISTRATION OF INDAPAMIDE, HYDRO-CHLOROTHIAZIDE AND CAPTOPRIL IN SPONTANEOUSLY HYPERTENSIVE RATS.

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Endothelial function is altered in essential hypertension and various antihypertensive drugs are able to restore normal function leading also to the improvement of vasorelaxation. Diuretics and ACE inhibitors belong to the antihypertensive substances with marked positive effect on endothelial function. The mechanism of action of different antihypertensive drugs which belong to the same group may however differ according to their structure and the dose.

The purpose of this study is to investigate the effect of chronic indapamide, hydrochlorothiazide, and indapamide+captopril treatment on the blood pressure and to compare chronic and acute effects of substances studied on the endothelial function in SHR by evaluating the endothelium-dependent relaxation of the femoral artery. Methods Chronic experiment: four groups of 6-week-old male SHR were orally treated with diuretics: indapamide (1mg/kg/day) or hydrochloro-thiazide (10mg/kg/day), or ACE inhibitor captopril (1mg/kg/day), and indapamide+captopril combination in the same dose as alone for 6 weeks. Systolic blood pressure was measured by the tail-cuff plethysmography every week. Acute experiment: femoral artery rings from 12-week-old SHR were pre-incubated by indapamide or hydrochlorothiazide or captopril or indapamide+captopril in the same concentration 10^{-4}mol/l.The endothelium-dependent relaxations were tested on femoral artery rings pre-contracted with serotonin (10^{-5}mol/l) using the Mulvany myograph. Relaxations were induced by acetylcholine (10^{-8}-10^{-5}mol/l). All drugs significantly decreased the blood pressure rise in young SHR. Chronic indapamide treatment significantly increased relaxation responses compared to the control group and hydrochlorothiazide group. Captopril treatment failed to affect the relaxation of the femoral artery. Indapamide+captopril combination increased relaxation responses similarly as indapamide alone. Regarding acute experiments, all substances studied increased significantly relaxation of femoral artery isolated from SHR. While indapamide increased relaxation responses of femoral artery in both acute and chronic experiments, hydrochlorothiazide and captopril failed to affect relaxation responses after chronic treatment. It seems that lower concentration of indapamide is enough to improve relaxation responses during the chronic treatment.

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THE ACTIVITY OF ARGINASE AND NITRIC OXIDE SYNTHASE IN BRONCHIAL HYPERREACTIVITY

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Nitric oxide (NO) has significant position in the regulation of various functions in the respiratory system. L-arginine is a basic substrate for NO biosynthesis. It is the precursor for the synthesis of different proteins, urea, polyamines, proline, glutamate, creatine or agmantine, too. The synthesis and catabolism of L-arginine are under control some enzymes e.g. two arginase isoenzymes and three NO-synthases. The metabolic fate of L-arginine is determined by the changes in the activity of these enzymes in the airways. We examined the effect of modulation of nitric oxide synthase (NOS) and arginase activity in guinea pig model of ovalbumine-induced bronchial hyperreactivity. Animals were pretreatment with nonselective (L-NAME – L-$N^{?}$-nitroarginine methylester) and selective (aminoguanidine) NOS inhibitor before the bronchial hyperreactivity provocation. Guinea pigs were killed twenty four hours after last allergen exposure and small strips from trachea and lung tissue were placed to organ chambers with Krebs-Henseleit solution. Preparations were contracted with cumulative doses of histamine and acetylcholine ($10^{-8} – 10^{-3}$ mol/l) after 60 minutes of the incubation. The arginase or nonselective arginase inhibitor (NOHA – N^{G}-hydroxy-L-arginine acetate) were used only in *in vitro* conditions (30 minute incubation of the tissue strips with agent) twenty four hours after last allergen exposure. The preparation were contracted after 90 minutes with the cumulative doses of histamine and acetylcholine ($10^{-8} – 10^{-3}$ mol/l), too. The modulation of the ovalbumine-evoked bronchial hyperreactivity was dependent on the type of used NOS inhibitor (selective, nonselective), type of administration (inhalation, intraperitoneal), used of bronchoconstrictor mediators and airways area (lung tissue, trachea). The arginase decreased the reactivity to histamine but increased (or was without effect) the reactivity to acetylcholine. The NOHA increased tracheal reactivity to acetylcholine only. We can conclude that used agents modulating the enzymatic systems related to NO influenced the bronchial hyperreactivity evoked by ovalbumine. We need further experiments to clarify the involvement of these enzymes in the bronchial hyperreactivity.

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MORPHOLOGICAL CONSEQUENCES OF NITRIC OXIDE DONOR ADMINISTRATION TO SHR FROM PREHYPERTENSIVE PERIOD THROUGH ADULTHOOD.

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Participation of nitric oxide (NO) in developing of pathological changes in SHR is not clear. Long-term administration of L-arginine and/or NO donor to adult SHR did not revealed any effect on blood pressure (BP), cardiac hypertrophy (heart/body weight ratio) and geometry of conduit arteries (1, 2). The aim of this study was to evaluate whether long-term administration of NO donor (pentaerythrityl tetranitrate – PETN) from prehypertensive period through adulthood prevent pathological changes in SHR. Four-week old Wistar rats and SHR were divided in three groups: 1) Wistar rats, 2) SHR, and 3) SHR administrated PETN p.o. 50 mg. kg^{-1} body weight in drinking water, twice daily by gavage in a total daily dose of 100 mg.kg^{-1} body weight. BP was measured by plethysmographic method on tail artery. After 6 weeks of treatment the animals were sacrificed and perfused by glutaraldehyde fixative under the pressure of 120 mm Hg. Thoracic aorta (AT), carotid (AC) and coronary artery (RS) were processed according to standard electron microscopic procedure. The wall thickness (WT) (tunica intima + tunica media), cross sectional area (CSA), inner diameter (ID), and WT/ID ratio were evaluated using light microscopy. At the end of the experiment BP was increased in SHR and it was not affected by PETN administration but administration of PETN evoked decrease of cardiac hypertrophy in SHR. Different effect of PETN was observed on conduit arteries. The most important parameter – arterial wall mass was, in comparison to SHR, increased in all three conduit arteries. In AT and AC other parameters was not influenced, in RS due to increase of ID was decreased WT/ID. In conclusion: Contrary to that data received

after PETN administration to adult SHR (2) we observed in young SHR preventive effect of PETN administration against cardiac hypertrophy and undesirable exaggerated effect on hypertrophy of the arterial wall in conduit arteries. PETN administration did not affect BP in both experiments. From these results we suggest that the deficiency of endogenous NO is probably not the main course for increase of BP with consequent pathological alterations in cardiovascular system of SHR.

1.Kristek F: Exp. Physiol., 83: 595-603, 1998
2.Kristek F, Fáberová V, Varga I: Physiol. Res. 52: 709-713, 2003.

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EFFECT OF PRAZOSIN ON FUNCTIONAL AND MORPHOLOGICAL CARDIOVASCULAR PARAMETERS IN YOUNG SPONTANEOUSLY HYPERTENSIVE RATS.

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The pathogenesis of essential hypertension is still not fully understood, but genetic factors and the increase activity of the sympathetic nervous system are likely to be involved. The aim of this study was to examine the effect of prazosin, an alpha$_1$-adrenergic receptor antagonist, on blood pressure and functional and structural changes in thoracic aorta in young spontaneously hypertensive rats (SHR). Four-week-old male SHR were taken into experiment; they were treated with prazosin (daily dose of 10mg/kg/day by a gavage) for 5 weeks. Systolic blood pressure (SBP) was measured by the tail plethysmografic method. Rings of thoracic aorta were mounted in organ baths for measurement of isometric contractile force. Morphological changes of thoracic aorta were measured using light microscopy. SBP in SHR (149±2mmHg) was higher than in age-matched controls (109±2mmHg). The increase in SBP in SHR was accompanied by higher heart weight/body weight (HW/BW) ratio (5.8±0.2) comparing to controls (4.6±0.3), indicating hypertrophy of the heart. At the end of prazosin treatment SBP (139±3mmHg) and HW/BW ratio (4.7±0.2) in SHR were significantly reduced. Reduction of SBP (101±4mmHg) as well as HW/BW ratio (3.9±0.1, $P<0.05$) was also observed in control Wistar rats. In phenylephrine-precontracted aortic rings from SHR acetylcholine-induced relaxation was inhibited at higher concentration of acetylcholine. Long-term administration of prazosin to SHR completely prevented the reduction of ACh-induced relaxation. The wall thickness (WT) and cross sectional area (CSA) of aorta in SHR were decreased in comparison to control group. In prazosin-treated Wistar rats WT, CSA and wall thickness/internal diameter ratio were significantly decreased but geometry of aorta in SHR was not significantly changed by prazosin. In conclusion, in SHR rats, prazosin administration prevented SBP elevation and exerted beneficial effect on regression of cardiac hypertrophy. Prazosin prevented impairment of endothelium-dependent relaxation but it had no significant effect on geometry of thoracic aorta.

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MORPHOLOGICAL STUDY OF THORACIC AORTA AND CAROTID ARTERY IN 3 AND 52 WEEK-OLD SHR AND HTG RATS

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SHR represent genetic model of hypertension and HTG rats represent an animal model of human metabolic syndrome X. The aim of the study was to compare morphological changes of thoracic aorta (AT) and carotid artery (AC) in 3-week-old (3w) and 52-week-old (52w) SHR and HTG with control Wistar rats. After sacrificing the rats were perfused with a glutaraldehyde fixative under the pressure 90 mm Hg (3w) and 120 mm Hg (52w) for 10 minutes via cannula placed into left ventricle. Middle parts of AT and AC were excised and processed according to standard electron microscopy procedure. Wall thickness (WT), inner diameter (ID), cross sectional area (CSA), and WT/ID ratio (WD) were evaluated in light microscopy. In 3w: blood pressure (BP) was increased only in HTG group in comparison to age-matched control Wistar rats. Hypertrophy of myocardium was in both experimental groups. WT and CSA in AT were decreased in both experimental groups, in AC only in HTG rats. ID was decreased in both arteries in both groups. WD of AT in SHR was decreased, in AC it was increased. WD of both arteries in HTG did not differ from the age-matched control Wistar rats. In 52w: BP was increased in both experimental groups in comparison to age-matched control Wistar rats, more markedly in SHR. Hypertrophy of myocardium was found only in SHR. WT of both arteries in both experimental groups was increased. CSA of both arteries in SHR was increased. In HTG rats CSA of AT did not differ from the controls and CSA of AC was decreased. ID of both arteries in SHR did not differ from controls, contrary to HTG rats where it was decreased in both arteries. WD was increased in both arteries of both groups in comparison to control Wistar rats. In conclusion, disproportion between development of BP and geometry of AT and AC in SHR and HTG rats were observed in two ontogenic periods. In 3w SHR were in prehypertensive period, but HTG had already elevated BP. In 52w BP of SHR was higher than in HTG. Alterations of BP were not in harmony with alterations in geometry of both arteries of SHR and HTG rats. We suggest that BP is not the main stimuli of changes during ontogenic development of SHR and HTG rats.

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LONG-TERM IMPACT OF PRAZOSIN ON GEOMETRY OF LARGE ARTERIES OF SHR AND WISTAR RATS

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We evaluated the effect of long-term administration of Alpha 1 receptor blockator Prazosin (Pra) to spontaneously hypertensive rats (SHR) from prehypertensive phase and to Wistar rats. Systolic blood pressure (SBP), body weight (BW), heart weight (HW), ratio HW/BW and geometry of conduit arteries - carotid artery (AC) and coronary artery (RS) were studied. Four weeks old rats were divided into 4 groups: 1) normotensive Wistar rats, 2) Wistar+Pra, 3) SHR, and 4) SHR+Pra. Prazosin was administered in daily dose 10 mg/kg by gavage. SBP was measured non-invasively by the plethysmographic method on the tail artery. After 5 weeks of administration rats were anaesthetised and perfused by glutaraldehyde fixative by a constant perfusion pressure 120 mm Hg. Conduit arteries (AC, RS) were processed for transmission electron microscopy. Wall thickness (WT) and inner diameter (ID) of AC and RS were measured by light microscopy. Cross sectional area (CSA) and wall thickness/inner diameter (WD) were calculated.

	Wistar	Wistar+Pra	SHR	SHR+Pra
SBP (mm Hg)	109±1.7	101±3.5 *	149±2.1 **	139±2.9 $^{+}$
HW/BW(mg/g)	4.6±0.3	3.9±0.1 *	5.8±0.2 **	4.5±0.2 $^{++}$
AC				
WT(μm)	27.5±0.8	23.4±1.1 **	35±0.7 **	34.8±1
ID (μm)	757±17.8	793±27.8	723±19	747±15.8
CSAx10^{3}μm^2	67.5±1.8	59.4±2.4 *	83.2±2 **	85.3±3
WDx10^{-2}	3.7±0.2	3±0.2 *	4.9±0.2 **	4.7±0.2
RS				
WT (μm)	10.4±0.6	11.1±0.4	14.5±0.7 **	13.4±2.1
ID (μm)	213±8.8	239±6.3 *	212±4.1	237±17
CSAx10^{3}μm^2	7.3±0.5	8.7±0.4 *	10.3±0.5 **	10.5±1.6
WD x 10^{-2}	5.1±0.6	4.7±0.2	7±0.4 **	6.1±1.3

* $p < 0.05$, ** $p< 0.01$ versus Wistar rats, $^{+}$ $p < 0.05$, $^{++}$ $p< 0.01$ versus SHR.

In conclusion, long-term blockade of Alpha 1 receptors with Prazosin reduced SBP and decrease HW/BW ratio in both SHR and Wistar rats. Prazosin did not affect geometry of carotid and coronary artery in SHR and evoked remodelling of conduit arteries of normotensive Wistar rats.
The study was supported by VEGA grant 2/3145/23, Slovakia.

CARDIOVASCULAR DISEASES AND MOLECULAR VARIANTS OF THE RENNIN-ANGIOTENSIN SYSTEM COMPONENTS IN THE SLOVAK POPULATION.

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Molecular variants of individual components of the rennin-angiotensin system (RAS) are reported to constitute the inherited predisposition to some cardiovascular diseases in human, e.g. essential hypertension, myocardial infarction, ischaemic heart disease or dilated cardiomyopathy. The frequency of these variants depends highly on the race and population. We examined the M235T molecular variant of the angiotensinogen gene and the I/D polymorphism of the ACE gene in Slovak healthy population, in patients with diagnosed essential hypertension, in patients who had undergone myocardial infarction, patients with diagnosed ischaemic heart disease and patients with dilated cardiomyopathy. DNA from 835 subjects was tested for the presence of M235T and I/D molecular variants. The frequency of both these polymorphisms in the Slovak population correlates with other Caucasian populations. In the group of hypertensive patients, the frequency of the M235T molecular variant was increased compared to controls, predominantly in males (0.45 vs. 0.28), while in the I/D polymorphism the incidence of the D allele was the same for both controls and hypertensives (0.49 vs. 0.50). A significant increase in the D allele frequency compared to the controls occurred in the group of infarcted patients (0.63), in group of patients with ischaemic heart disease (0.60) and also in patients with dilated cardiomyopathy (0.59). The increased frequency of the M235T allele in hypertensive patients compared to the healthy population confirms that the M235T variants is associated with increased blood pressure in the Slovak population. In the Slovak poulation, I/D polymorphism of the ACE gene is associated with myocardial infarction, ischaemic heart disease and dilated cardiomyopathy, rather than with hypertension. We conclude, that in the Slovak population D alelle of ACE gene polymorphism represents a risk factor for several cardiovascular diseases.
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HIGH-RESOLUTION ELECTROCARDIOGRAPHY OF P WAVE IN PATIENTS WITH PAROXYSMAL ATRIAL FIBRILLATION AND PATIENTS WITH ARTERIAL HYPERTENSION AND DIABETES MELLITUS.

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Non-invasive prediction of atrial fibrillation is more problematic in comparison with prediction of ventricular arrhythmias. High-resolution ECG of P wave can determine delay atrial conduction predisposing to atrial fibrillation. Filtered P wave duration was measured on signal averaged ECG to determine the duration of atrial electrical activation. Ventricular conduction was also measured. Left atrium diameter was assessed by echocardiography. Our study group consists of 21 patients with history of documented atrial fibrillation and sinus rhythm during examination (group 1). Other group consist of 16 patients without history of atrial fibrillation, but with arterial hypertension and diabetes mellitus (group 2), because these combination seems to be more strongly associated with atrial fibrillation. Duration of filtered P wave was 138.8 ± 13.48 ms in group 1 (mean noise level 0.51 μV) and 124.9 ± 11.36ms in group 2 (noise level 0.48 μV). Duration of filtered QRS complex was 112.0 ± 10.49 ms in group 1 (noise level 0,33 μV) and 110.5 ± 9.62 ms in group 2 (noise level 0,36 μV). Diameter of left atrium in parasternal long axis projection was 43.5 ± 6.58 mm in group 1 and 37.2 ± 4.03 mm in group 2. We found out prolonged atrial conduction in all patients with history of atrial fibrillation independently on time of examination from its last paroxysm. Duration of signal averaged P wave in hypertonic diabetic patient achieved upper limit of normal value (125ms in most studies). Tree patient without history of atrial fibrillation had delay atrial conduction. Duration of QRS complexes was similar in both groups. Left atrium diameter correlated with its electric conduction. High-resolution electrocardiography can identify non-invasively electric properties of myocardium, which are essential for reentry mechanism of arrhythmias.

CAN NITRIC OXIDE (NO) INFLUENCE THE HIGHER BRAIN FUNCTIONS?

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It was suggested that NO may affect the basic memory processes. In the last 15 years the memory-guided saccade (MGS) task has been used in humans in order to study the short-term spatial memory. In this task a subject fixates a central point in the visual field and continues fixating it while a visual stimulus is flashed briefly in the visual periphery. Subject has to remember the location of the peripheral visual stimulus. After the central fixation point is extinguished he/she has to make a saccade to the remembered location. We were interested in question whether the Provinol, which is known to increase the activity of NO syntase, can influence the performance of subjects in the MGS task. Two groups of healthy students participated in examinations. At the beginning the visually-guided saccade (VGS) task was introduced in order to obtain the control values. Following it the MGS task was introduced.This procedure was repeated twice: 2 and 3 hours after the first session. In the first group the Provinol was administered immediately after the end of the first session (4 mg/kg of body weight). The second group underwent the examinations without Provinol. The saccadic accuracy did not differ in all the three VGS measurements. A slight tendency to lower SD values of saccadic amplidudes was seen 2 hours after the Provinol administration. The better saccadic accuracy, the significantly lower number of inaccurate memory-guided saccades as also the lower standard deviation values of the saccadic amplitudes were recorded 2 hours after the Provinol administration. The significant increase of saccadic inaccuracy with accompanying higher values of the standard deviations of the eye movement amplitudes were recorded 3 hours after the Provinol administration. Thus, the Provinol influenced positively the performance of the subjects in the MGS task performed two hours after its administration but did not affect it 3 hours after administration. It is known the MGS paradigm comprises 3 successive phases: 1. perception, 2. memorization and 3. programming and starting the saccade (1). The question remains which of the above phases are affected by the Provinol.

(1) Pierrot-Deseilligny, Ch. et al., Brain, 126, 2003, 1460-1473

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