Session I. NITRIC OXIDE IN SALT HYPERTENSION

THE BALANCE OF NITRIC OXIDE AND PRESSOR SYSTEMS IN VARIOUS FORMS OF EXPERIMENTAL HYPERTENSION IN THE RAT

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The balance between the principal depressor system (nitric oxide - NO) and main pressor systems (renin-angiotensin system - RAS, sympathetic nervous system - SNS) was studied in two different models of experimental hypertension - salt hypertensive Dahl rats and rats made hypertensive by chronic NO synthase inhibition by L-NAME. The animals were subjected to consecutive acute blockade of RAS (captopril 10 mg/kg i.v.) and SNS (pentolinium 5 mg/kg) which was followed by acute bolus of L-NAME (30 mg/kg) and aminoguanidine (inducible NOS inhibitor, AMG 50 mg/kg). Both forms of hypertension are characterized by augmented pentolinium-induced BP fall compared to the respective controls, whereas depressor response to acute captopril injection was slightly increased in L-NAME hypertensive rats only. On the other hand, BP elevation after acute L-NAME injection was not significantly enhanced in salt hypertensive Dahl rats. The same was true for L-NAME hypertensive rats, if their NO synthase was acutely inhibited by AMG. Although total BP rise induced by L-NAME plus AMG administration was comparable in normotensive and hypertensive rats, there was a relative deficit of depressor systems to compensate the augmented activity of pressor systems. It is evident that both apparently contrasting hypertensive models are characterized by 1) the enhanced contribution of SNS to BP maintenance, and 2) the relative NO deficiency facing the balance of Normal and Pathological Physiology SAS, Bratislava, Slovakia. No differences in the principal depressor system (nitric oxide NO) and main pressor systems (renin-angiotensin system RAS, sympathetic nervous system SNS) were demonstrated in Dahl salt-sensitive rats. Moreover, we have previously demonstrated that chronic excess salt intake causes a substantial long-term elevation of blood pressure (BP) in salt-sensitive Dahl rats. More severe forms of hypertension were observed if immature (weanling or prepubertal) rats were exposed to high salt intake. On the other hand, similar hypertensive stimuli applied to adult animals cause less pronounced, benign forms of salt hypertension. It should be noted that the difference in the response of young and adult rats to high salt intake is not only in the degree of hypertension but also in the participation of particular pressor and depressor systems. From our previous data it is evident that the impaired balance between the vasoconstrictor (RAS, SNS) and vasodilator systems (NO) is present in salt hypertension of Dahl rats. Sympathetic nervous system (SNS) is more important for the development and maintenance of salt hypertension in immature rats, whereas depressor systems (such as vasopressin or angiotensin II) are involved especially in the hypertensive response of adult animals. This is in agreement with our hypothesis on the different role of principal (SNS) and reserve (NO synthetase) systems in salt hypertension of young and adult animals.

OXYGEN RADICALS IN RATS WITH NO-DEFICIENT HYPERTENSION OR SALT HYPERTENSION

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Reactive oxygen and nitrogen species play an important role in the maintenance of vascular homeostasis. Alterations in the rate of radical formation and in the extent of their scavenging have been implicated in vascular dysfunction seen in atherosclerosis, diabetes, hypertension, etc. The evanescent nature of reactive species has made their measurement rather problematic. In our recent study we have used the detection of superoxide anions by means of lucigenin chemiluminescence. The aim of our study was to evaluate the production of radicals in aorta rings of Wistar rat with L-NAME hypertension and Dahl rat with salt hypertension, respectively. We found higher luminescence (by 30%) in aortas of L-NAME hypertensive rat in comparison with controls. This form of experimental hypertension could be partly normalized by chronic drinking of N-acetylcysteine solution (20 g/l) for 30 days. We found a similar increase of luminescence in the aortas of salt-sensitive Dahl rats with salt hypertension. Surprisingly, greater radical production in thoracic aorta was observed in adult than in young salt hypertensive animals, although acute superoxide removal by tempol administration lowered blood pressure more in young salt hypertensive Dahl rats. No differences in superoxide chemiluminescence were detected in renal homogenates measured under basal conditions (without NADH supplementation). Our future effort will be focused on the determination of NADH/Pi/H oxidase activity in the kidney and blood vessels of rats with various forms of experimental hypertension. Our findings support the role of reactive oxygen species in the pathogenesis of particular forms of experimental hypertension. This study was supported by research grant No. 305/03/0769 from the Grant Agency of the Czech Republic and AVOZ 5011922.

LESS IMPORTANT ROLE OF ENDOTHELIN IN SALT HYPERTENSION OF YOUNG THAN ADULT DAHL RATS

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We have previously demonstrated that chronic excess salt intake causes a substantial long-term elevation of blood pressure (BP) in salt-sensitive Dahl rats. More severe forms of hypertension were observed if immature (weanling or prepubertal) rats were exposed to high salt intake. On the other hand, similar hypertensive stimuli applied to adult animals cause less pronounced, benign forms of salt hypertension. It should be noted that the difference in the response of young and adult rats to high salt intake is not only in the degree of hypertension but also in the participation of particular pressor and depressor systems. From our previous data it is evident that the impaired balance between the vasoconstrictor (RAS, SNS) and vasodilator systems (NO) is present in salt hypertension of Dahl rats. Sympathetic nervous system (SNS) is more important for the development and maintenance of salt hypertension in immature rats, whereas depressor systems (such as vasopressin or angiotensin II) are involved especially in the hypertensive response of adult animals. The aim of our study was to investigate whether the involvement of endothelin-1 in the pathogenesis of salt hypertension in Dahl rats is also age-dependent. We have performed two types of studies in Dahl rats in which we have examined the effects of acute or chronic administration of ETA receptor antagonist BDF208075 on salt hypertension development and/or maintenance in young (high salt intake from weaning at the age of 4 weeks) and adult (high salt intake from the age of 12 weeks). Both young and adult rats with established salt hypertension are characterized by a modest participation of endothelin in the actual BP maintenance. On the other hand, the role of endothelin in the development of salt hypertension is strictly age-dependent because chronic ETA receptor blockade prevented hypertension in adult but not in young animals. This is in agreement with our hypothesis on the different role of principal (SNS) and reserve (NO synthetase, vasopressin, endothelin) pressor systems in salt hypertension of young and adult animals. Partially supported by grants 305/03/0769 and AVOZ 5011922.

POLYMORPHISM OF INDUCIBLE NO SYNTHASE IN DAHL RATS

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Nitric oxide synthase (NOS) genes are candidate genes for genetic hypertension. Various polymorphisms of inducible NOS (Nos2) gene were demonstrated in Dahl salt-sensitive rats (1,2). We have analyzed the polymorphism of this gene in Prague colony of Dahl rats. Adult salt-sensitive (S) and salt-resistant (R) Dahl rats from the colony of the Institute of Physiology AS CR were used. This colony was established from the initial breeding pairs provided by Prof. J.P. Rapp in 1986. Genomic DNA was obtained from liver tissue by using of phenol extraction and ethanol precipitation. Specific primers for PCR were synthesized according the above mentioned papers. PCR products were analyzed by electrophoresis on 2% agarose gel and visualized by using of ethidium bromide staining. No polymorphism between salt-sensitive and salt-resistant Dahl rats was found in Nos2 gene fragment defined by gene-specific primers from the paper of Deng and Rapp (1). Moreover, two-step PCR procedure according to Chen et al. (2) did not disclose any difference between S and R rats in restriction site for Piel restriction endonuclease. In conclusion, we have not found any polymorphism in Nos2 gene in Prague colony of Dahl rats. Our results clearly demonstrated the differences among Dahl rats of our own colony and those of colonies from Harlan and Toledo. Supported by AVOZ and research grant 305/03/0769 (GA CR).


Session II. NITRIC OXIDE IN SPONTANEOUS HYPERTENSION

The role of membrane lipids in ion transport alterations in the erythrocytes

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Changes of ion transport across erythrocyte membrane are associated with salt hypertension in Dahl rats. This is also accompanied with elevations of plasma triglyceride and total cholesterol levels. The aim of the present study was to search for the relation between abnormalities of ion transport and lipid composition of erythrocyte membrane in Dahl rats. Erythrocytes of salt hypertensive Dahl rats are characterized by increased membrane phospholipids (sphingomyelin, phosphatidylinositol and phosphatidylyserine). Membrane cholesterol participates in the regulation of Na-K pump activity, Na+–K+ cotransport rate and Rb+ (K+) leak in salt-hypertensive Dahl rats. On the other hand, total phospholipids (particularly via sphingomyelin or phosphatidylinositol and phosphatidylyserine) participate in the regulation of erythrocyte Na+ content, ouabain-sensitive Rb+ (K+) transport and Na+–K+ cotransport. In contrast, no relationship of membrane phospholipidethanolamine and phosphatidylcholine content to ion transport was found. Our study confirmed the importance of membrane lipid composition in the control of cellular ion transport. It is evident that particular classes of membrane lipids may exert distinct effects on different ion transport pathways. Partially supported by grant 305/03/0769 and AVOZ 5011922

CALCIUM INFLUX IN EXPERIMENTAL HYPERTENSION: NIFEDIPINE-SENSITIVE BLOOD PRESSURE COMPONENT

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Elevated cytosolic free calcium plays a key role in vascular smooth muscle contraction. Its increase can be achieved by different mechanisms, but enhanced Ca2+ influx through voltage-dependent Ca2+ channels (VDCC) was suggested to be involved in the pathogenesis of various forms of hypertension. We have therefore tried to determine the contribution of VDCC to the maintenance of blood pressure (BP) in particular forms of experimental hypertension, especially in salt hypertension of Dahl rats and in NO-deficient hypertension induced by chronic L-NAME treatment. The acute administration of nifedipine (0.5 mg/kg iv) or verapamil (2 mg/kg iv) in conscious chronically cannulated rats was used to estimate VDCC-dependent BP reduction. Our further effort was focused to elucidate the relationship between nifedipine-sensitive BP-suppressing and agonist-dependent BP components, i.e. whether nifedipine administration lowers BP effects of endogenous or exogenous catecholamines and angiotensin. Nifedipine reduced BP by 67±8 mm Hg in salt hypertensive Dahl rats and by 61±7 mm Hg in L-NAME hypertensive rats (vs. 30±3 and 18±3 mm Hg in the respective controls). Similar data were obtained after verapamil injection. Furthermore, our results indicate that BP reduction caused by acute VDCC blockade was proportional to basal BP values (Dahl rats: r = 0.95, n=36; L-NAME rats: r = 0.91, n=15, p=0.001 both). On the contrary, the residual (nifedipine-insensitive) blood pressure was almost independent of initial BP levels. It should also be noted that nifedipine or verapamil administration almost completely prevented BP rise elicited by acute L-NAME injection. It can be concluded that nifedipine-sensitive BP component seems to be responsible for the major part of BP elevation seen in the above mentioned forms of experimental hypertension. Our results fully confirm the importance of enhanced Ca2+ influx through voltage-dependent Ca2+ channels in the pathogenesis of high blood pressure. Partially supported by grants 305/03/0769, 305/02/P606

THE EFFECT OF TEMPOL ON CELL CALCIUM HANDLING IN VSMC ISOLATED FROM SHR

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Both greater basal free cytosolic calcium concentration ([Ca2+]i) and increased [Ca2+]i response to angiotensin II (Ang II) have been previously demonstrated in aortic vascular smooth muscle cells (VSMC) in male spontaneously hypertensive rat (SHR). Furthermore, aortas from male SHR were characterized by enhanced superoxide formation. Therefore we have investigated the influence of oxidative stress reduction by means of tempol, a superoxide dismutase (SOD) mimetic, on basal free cytosolic calcium concentration and [Ca2+]i response to angiotensin II. SHR male (n=10) and WKY male (n=10) rats aged 10 weeks were used in this study. [Ca2+]i was measured by image analysis of single myocytes (n=840) loaded with Fura-2. Confluent primary cultures were used. The effect of tempol (10−4M, 10−3M, 10−2M) on resting [Ca2+]i, and on [Ca2+]i response to angiotensin II has been evaluated in VSMC isolated from hypertensive and normotensive rat strains. In the presence of SOD mimetic, tempol, basal [Ca2+]i was not significantly altered in VSMC from normotensive rats, but in the cells isolated from SHR, basal [Ca2+]i was reduced to levels similar to those isolated from WKY males. Furthermore, tempol attenuated angiotensin II-stimulated [Ca2+]i response more in VSMC isolated from male SHR than from normotensive controls. These results suggest that increased [Ca2+]i level in VSMC isolated from SHR is dependent on the increased amount of superoxide anions in SHR. Supported by research grant 305/02/P606 from the Grant Agency of CR and is a part of research project AVOZ 5011922.
pulmonary arteries. In phenylephrine ω was not effected by the removal of vascular endothelium. Also, treatment melatonin caused a concentration-dependent relaxation. This response field stimulation of perivascular nerves were slightly inhibited by only Neurogenic contractions of main pulmonary artery induced by electrical oxide synthase activity in endothelium of large pulmonary arteries. treatment had no significant effect on acetylcholine-induced relaxation of animals was not significantly different from that of SHR. Melatonin change the magnitude of melatonin-induced relaxation. The magnitude differences in this respect were observed in Mols group (both were matrix in arterial wall in PETN group did not differ from the SHR. It means that increase of extracellular matrix (CSA), and WT/ID ratio. These findings are in a good agreement with our earlier observation on conduit arteries of SHR after long-term PETN administration. Contrary to this in Mols group ID and CSA were decreased. Nevertheless, HW/BW ratio was in 17w and 52w old HTG rats increased (no difference was found between 3w groups). The geometry of both TA and CA revealed that WT of HTG rats, contrary to control, during the ontogeny progressively increased – WT in 3w old HTG rats was decreased, but in 17w old HTG rats its value was decreased only in TA, and in 52w old HTG rats WT was increased in both arteries. In comparison to control CSA and ID of both HTG arteries were decreased in all ontogenic periods. WT/ID ratio was increased in CA in 17w and 52w old HTG rats; in TA it was increased only in 52w old HTG rats. Hypertiglycericemia in rats evoked changes in geometry of conduit arteries. Taking into account Laplace’s law, the changes in the arterial geometry exert a negative effect on physiological parameters and functional consequences lead to negative effects on supply of nutritional demand of the respective areas. Support: VEGA 2/3145/23, Slovakofarma, J.S.C..

**LONG-TERM EFFECT OF NITRIC OXIDE DONORS ON GEOMETRY AND ULTRASTRUCTURE OF BASILAR ARTERY OF SHR**

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The aim of the study was to compare the geometry of conduit arteries of SHR treated by PETN (200 mg/kg b.w./day, p.o, for 6 weeks), (3) SHR treated by Mols (100 mg/kg b.w./day, p.o.). The experiment lasted 6 weeks. Blood pressure (BP) was measured by the tail plethysmographic method. At the end of the experiment the rats were perfused with glutaraldehyde fixative under the pressure of 120 mm Hg. The basilar artery was excised and processed according to standard electron microscopy procedure. Geometry of the artery was measured on semithin sections in the light microscopy. Volume densities of cellular and extracellular components of the arterial wall (tunica intima and tunica media) were estimated on ultra-thin sections quantitatively using the point counting method. No differences in BP and heart rate were observed among the groups. In both PETN and Mols groups the body weight and heart weight were lower than in SHR group. Nevertheless, the heart weight/body weight ratio was decreased (p<0.01) only in the Mols group. In comparison to SHR we did not observe in PETN group changes in inner diameter (ID), wall thickness (WT), cross sectional area (CSA), and WT/ID ratio. These findings are in a good agreement with our earlier observation on conduit arteries of SHR after long-term PETN administration. Contrary to this in Mols group ID and CSA were significantly increased. Volume densities of cellular and extracellular matrix in arterial wall in PETN group did not differ from the SHR. Differences in this respect were observed in Mols group (both were increased but predominantly extracellular matrix). It means that increase of arterial wall mass (CSA) in the Mols group was substantially due to increase of extracellular matrix. Our results suggest that long-term administration of NO donors to SHR did not evoke beneficial effect on BP, heart rate and geometry and structure of basilar artery. Thus, that the pathological changes in cardiovascular system of SHR are not evoked by the deficiency of endogenous NO production. Support: VEGA 2/3145/23, Slovakofarma, J.S.C. Hlohovec.

**GEOMETRY OF CONDUIT ARTERIES OF HYPERTRIGLYCERICERIDEMIC RATS DURING ONTOGENIC DEVELOPMENT**

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The aim of this study was to compare geometry of the conduit arteries of HTG rats with control Wistar rats during ontogenic development. HTG rats and age-matched controls of three ontogenic periods were taken for the study: 3 weeks old rats (3w), 17 weeks old rats (17w) and 52 weeks old rats (52w). Blood pressure (BP) was measured by the tail plethysmographic methods. After sacrificing by overdose of anaesthesia the animals were perfused via left ventricle with a glutaraldehyde fixative under the pressure of 120 mmHg. Middle part of the thoracic aorta (TA) and middle part of the carotid artery (CA) were excised and processed according to standard electron microscopy procedure. The arteries were cut perpendicularly to longitudinal axis. Geometry of the arteries -wall thickness (WT) and inner diameter (ID) were measured in light microscopy on semithin sections. Cross sectional area (CSA) and WT/ID were calculated. In comparison to age-matched controls BP was in all ontogenic periods of HTG rats increased and both HW and BW were decreased. Nevertheless, HW/BW ratio was in 17w and 52w old HTG rats increased (no difference was found between 3w groups). The geometry of both TA and CA revealed that WT of HTG rats, contrary to control, during the ontogeny progressively increased – WT in 3w old HTG rats was decreased, but in 17w old HTG rats its value was decreased only in TA, and in 52w old HTG rats WT was increased in both arteries. In comparison to control CSA and ID of both HTG arteries were decreased in all ontogenic periods. WT/ID ratio was increased in CA in 17w and 52w old HTG rats; in TA it was increased only in 52w old HTG rats. Hypertiglycericemia in rats evoked changes in geometry of conduit arteries. Taking into account Laplace’s law, the changes in the arterial geometry exert a negative effect on physiological parameters and functional consequences lead to negative effects on supply of nutritional demand of the respective areas. Support: VEGA 2/3145/23, Slovakofarma, J.S.C.

**NITRIC OXIDE SYNTHASE ISOFORMS IN PRAGUE HYPERTRIGLYCERICERIDEMIC RATS (HTG): GENE POLYMORPHISMS, PROTEIN EXPRESSION AND PARTICIPATION IN BLOOD PRESSURE REGULATION**

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Nitric oxide (NO) exerts a fundamental role in the regulation of cardiovascular and renal function. Three distinct nitric oxide synthase isoforms (NOSs) exist in mammalian cells: endothelial (eNOS), neuronal (nNOS) and inducible (iNOS). We have investigated possible polymorphisms for these NOSs between HTG and control rats (Lewis), but no mutation for any NOS isoform was detected. Currently, we are studying protein expression (Western blot) of particular NOS isoforms in left ventricle and aorta of both strains. In order to reveal the role of NO produced by the above NOSs in blood pressure (BP) maintenance we have used three different NOS inhibitors in conscious chronically cannulated rats in which pressor systems (RAS and SNS) were acutely blocked by losartan and pentolinium, respectively. There was no strain difference in BP response to L-NAME (30 mg/kg i.v.) which inhibits almost completely eNOS. On the contrary, we have observed enhanced BP response to dimethylguanidine (combined eNOS and iNOS inhibitor, 50 mg/kg i.v.) in HTG rats (+116±40 mmHg) vs. +81±54 mmHg). Forthcoming experiments should confirm the upregulation of iNOS in HTG by means of a more specific iNOS inhibitor – aminoguanidine. We also intend to evaluate the contribution of nNOS using S-methyl-L-thiocitrulline. Our results illustrate the complexity of the relationship between genetic factors and physiological parameters. Supported by grants no. 305/03/0769 (GAČR).

Session III. NITRIC OXIDE IN HEREDITARY HYPERTRIGLYCERIDEMIC RATS
Hypertriglyceridemia in rats appears to be associated with enhancement of systolic blood pressure and impairment of endothelium-dependent relaxation of isolated thoracic aorta. The objective of this study was to investigate the effect of long-term (4 weeks) treatment of hereditary hypertriglyceridemic (hHTG) rats with three drugs which, according to their mechanism of action, may be able to improve the endothelial function: simvastatin (10mg/kg/d), spironolactone (200mg/kg/d) and L-arginine (1g/kg/d). Systolic blood pressure was measured indirectly by tail-cuff plethysmography each week. Aortic endothelial nitric oxide synthase (eNOS) protein expression was determined by Western blot analysis. At the end of 4th week treatment blood pressure in control hHTG group was 148±2 mm Hg and in control normotensive Wistar group 117±3 mm Hg. Arginine did not significantly influence blood pressure, but after simvastatin and spironolactone blood pressure was significantly lowered to the levels observed in control Wistar rats. In isolated phenylephrine-precontracted aortic rings from hHTG rats endothelium-dependent relaxation was diminished. Simvastatin improved acetylcholine-induced relaxation, but spironolactone and L-arginine did not significantly change endothelial function of the thoracic aorta. Western blot analysis revealed a decrease in aortic endothelial nitric oxide synthase (eNOS) protein levels in hHTG rats. Aortic eNOS protein expressions were normalized in simvastatin and spironolactone-treated groups but not in L-arginine-treated group. We conclude that long-term treatment of HHTG rats with simvastatin normalizes systolic blood pressure and NO-mediated relaxation of the thoracic aorta probably due to enhancement of endothelial NO production. Supported by VEGA grants No. 1/0532/03, 2/1166/23 and 2/3285/23.

ENDOTHELIAL FUNCTION OF CONDUIT ARTERY IN NEWBORN RATS WITH HEREDITARY HYPERTRIGLYCERIDEMIA
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It has been shown that endothelium derived nitric oxide plays an important role in regulation of vascular tone in the prenatal and early postnatal period. Adult hereditary hypertriglyceridemic (hHTG) rats are characterized by elevation of systolic blood pressure and impairment of endothelium-dependent relaxation of thoracic aorta. The aim of this study was to determine the reactivity and accompanying structural changes in thoracic aorta from 3-week-old hereditary hypertriglyceridemic rats. Rings of isolated thoracic aorta were mounted in organ baths for measurement of isometric contractile force. Morphological changes of thoracic aorta were measured using light microscopy. Systolic blood pressure in hHTG rats (109±2 mmHg) was slightly higher than that of age matched control rats (95±4 mmHg), P<0.05. The heart weight/body weight ratio was 5.39±0.09 vs. 4.36±0.25 in controls (P<0.01), indicating hypertrophy of the heart. Endothelium-dependent relaxation to acetylcholine and maximal isometric contraction of thoracic aorta to noradrenaline were not significantly different, but there was a rightward shift in the concentration-response curve to noradrenaline in aortic rings from hHTG rats. The values of wall thickness and cross sectional area of thoracic aorta were significantly decreased in comparison to control group. No difference was observed in wall thickness/inner diameter ratio. In conclusion, 3-week-old hHTG rats had elevated systolic blood pressure, cardiac hypertrophy, decreased wall thickness and cross sectional area of thoracic aorta but the endothelial function of this vessel was altered. J. Török et al.: Ann. N.Y.Acad.Sci. 976:469-457, 2002; Supported by grants VEGA No.2/3145/23 and 2/3166/23.

ROLE OF NITRIC OXIDE IN HYPOTHESIS AND ISCHEMIA
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Adaptation of rats to intermittent high altitude (IHA) hypoxia increases the tolerance of their hearts to all major manifestations of acute ischemia/reperfusion injury. The mechanism of this protective effect remains still unclear. The aim of our study was to analyze the possible role of nitric oxide (NO) and reactive oxygen species (ROS) in the antirarrhythmic protection by IHA hypoxia. Adult male Wistar rats were exposed to IHA hypoxia of 5000 m in a barochamber (4 h/day, 5 days/week, 24-32 exposures). A control group was kept under normoxic conditions (200 m) for the same period of time. The severity of ventricular reperfusion arrhythmias was assessed by a 5-point score on isolated perfused hearts after 15-min regional ischemia. NO synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME, 200 µmol/l), NO donor 5-nitrosoglutathione (GSNO, 10 µmol/l) and ROS scavengers tempol (1 mmol/l) or melatonin (10 µmol/l) were added to the perfusion solution 5 min before ischemia and were present throughout reperfusion. Concentration of NO and its oxidation products (nitrates, nitrites) in the coronary effluent was measured by a chemiluminescence method. Parallel groups of animals were used for immunohchemical detection of constitutive and inducible isoforms of NO synthase (eNOS and iNOS, respectively). In the normoxic group, the severity of reperfusion arrhythmias was significantly higher (score 4.04 ± 0.27) as compared with chronically hypoxic hearts (1.58 ± 0.38). L-NAME markedly reduced arrhythmias in controls (0.87 ± 0.28) but had no additional protective effect in the hypoxic group. In contrast, GSNO did not influence arrhythmias in controls but significantly increased the arrhythmia score in hypoxic animals (3.90 ± 0.42). Tempol and melatonin reduced reperfusion arrhythmias in the normoxic group (2.46 ± 0.69, 2.82 ± 0.58; respectively) and completely abolished arrhythmic protection in the hypoxic hearts (3.73 ± 0.51, 4.00 ± 0.32, respectively). IHA hypoxia increased myocardial expression of iNOS whereas the abundance of eNOS was reduced. Peak concentration of NO in the coronary effluent from reperfused hearts did not differ between the groups but the total production appeared to be increased in the IHA group. Our results suggest that endogenous NO contributes to antifusion ventricular arrhythmias in isolated hearts. Exogenous NO in controls, but not of chronically hypoxic rats; this difference cannot be explained by lower NO production by the hypoxic hearts. Exogenous NO is however proarrhythmic in the latter group. ROS appear to have a dual effect on cardiac susceptibility to arrhythmias: they are proarrhythmic in controls but play an essential role in the antirarrhythmic mechanism of chronic IHA hypoxia. Supported by GA CR 305/01/0279.

ROLE OF REACTIVE OXYGEN SPECIES IN CARDIOPROTECTION CONFERRED BY ADAPTATION TO CHRONIC HYPOXIA IN RATS
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Long-term adaptation of rats to intermittent high altitude (IHA) hypoxia increases cardiac tolerance to subsequent acute ischemic injury. Chronic hypoxia is also associated with increased production of reactive oxygen species (ROS), which may be implicated in cardioprotection. Therefore, our aim was to examine whether ROS generated during adaptation to chronic hypoxia and during acute ischemia/reperfusion insult play a role in the mechanism of improved cardiac ischemic tolerance. Adult male...
Wistar rats were exposed to IHA hypoxia of 7000 m in a hypobaric chamber for 8 h/day, 5 days a week; the total number of exposures was 25-32. One day after the last exposure, anesthetized open-chest animals were subjected to 20-min LAD coronary artery occlusion and 3-h reperfusion for infarct size determination (TTC staining). Antioxidant treatment was performed either (i) during the adaptation period by subcutaneous administration of N-acetylcysteine (NAC, 100 mg/kg) daily before the hypoxic exposure (chronic treatment) or ii) before test ischemia by a single dose of 4-hydroxy-TEMPO (tempol, daily before the hypoxic exposure (chronic treatment) or ii) before test reperfusion for infarct size determination (TTC staining). Antioxidant treatment prevented this decrease but it had no effect in the normoxic group. IHA hypoxia reduced the infarct size from 56.7 ± 4.5 % of the area at risk in the normoxic controls to 27.7 ± 4.9 % (p<0.05). Chronic reduction of ROS production by NAC treatment decreased the infarct size in the normoxic controls to 42.0 ± 3.4 % (p<0.05) but, on the other hand, it partially abolished protection induced by chronic hypoxia (to 41.1 ± 4.9 %; p<0.05). Acute administration of tempol had only minor cardioprotective effects in both normoxic and hypoxic groups. Our results suggest that ROS may play a dual role in myocardial susceptibility to acute ischemia/reperfusion injury: they contribute to low ischemic tolerance of control hearts but are also involved in the protective mechanism which develops during adaptation to chronic hypoxia. Supported by GA CR 305/01/0279.

MYOCARDIAL ISCHAEMIC TOLERANCE IS MODULATED IN NO DEFICIENT HYPERTENSIVE RATS
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NO has been implicated in the mechanisms of cardioprotection, however, the impact of NO deficiency (NOD) on ischemic tolerance of the heart is unclear. Our aim was to investigate the effect of chronic NOD on myocardial function and sensitivity to ischemia. Male adult Wistar rats were treated with L-NAME (40 mg/kg/day in drinking water) and after 4 weeks of treatment, hypertension and LV hypertrophy developed (blood pressure 165 ± 2.6 mm Hg; LV/BW index 1.61 ± 0.06 vs. 116 ± 2.5 mm Hg and 1.1 ± 0.03 in the controls, respectively; p<0.05). Heart rate, left ventricular developed pressure (LVDP), peak rate of pressure development (+dP/dtmax), left ventricular end-diastolic pressure (LVEDP) and coronary flow (CF) were used to assess myocardial function in isolated Langendorff-perfused hearts. Ischemic tolerance was tested by subjecting the hearts to test ischemic challenge (TI; 20 min global ischemia followed by 40 min reperfusion). Cardiac function was not changed in NOD hearts, however, their CF was markedly decreased (9.9 ± 0.6 vs. 13.6 ± 0.5 mL/min/g in the controls; p<0.05). After TI, maximal recovery of LVDP and +dP/dtmax in the normotensive group reached 34.8 ± 5.4% and 50.5 ± 4% of the initial pre-ischemic values. In contrast, NOD hearts showed an improved recovery of systolic function (LVDP 66.9 ± 7.9%, +dP/dtmax 79.4 ± 9.1%; p<0.05) and CF (94 ± 7% vs 69 ± 5% in controls; p<0.05), as well as attenuation of diastolic dysfunction (LVDP 6.5 ± 2.7 mm Hg vs 24.1 ± 7.9 mm Hg in the controls; p<0.05) and reduced severity of reperfusion-induced arrhythmias (arrhythmia score 3.1 ± 0.3 vs 4.3 ± 0.3 in the controls; p<0.05). Cardioprotective effects were reversed by blockade of mitochondrial K(ATP) channels with their selective inhibitor 5-hydroxydecanoate that did not influence control hearts. In addition, Western blot analysis of hypertrophy-related extracellular signal regulated kinases (ERK1/2) revealed enhanced activity of ERKs in NOD hearts after TI as compared to the controls. Conclusions: despite developing hypertension, hypertrophy and impaired myocardial perfusion, NOD hearts appear to be more resistant to ischemia/reperfusion injury. Potential factors contributing to enhanced ischemic tolerance in this model, except reduced generation of reactive species upon reperfusion, might be related to activation of adaptive mechanisms induced by oxidative stress and/or hypertrophic stimuli. The study was supported by grants VEGA 2/2063/22 and APVT 51-013802.

Session V. NITRIC OXIDE AND NATURAL POLYPHENOLS

REDUCTION OF EXPERIMENTAL ENDOTHELEMA BY RED WINE POLYPHENOLS
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Protective effects of red wine polyphenol compounds on cardiovascular system have been documented in numerous animal experimental as well as in human studies. These effects include vascular relaxation, antimicrobial and antioxidant activity. Endothelium protective effect of polyphenolic compounds isolated from red wine were studied in male Wistar rats administered 0.5 ml of CCl4/kg body weight intraperitoneally twice a week for 8 weeks. Endothelium (endothelial cells/10 ul of blood) was used as the marker of endothelial injury in vivo. Chronic CCl4 treatment for 8 weeks lead to a 3-fold increase of free endothelial cells in blood when compared to the baseline values (2.5±0.3). Parallel oral administration of red wine polyphenols 40 mg/kg/day significantly decreased the endothelium. Polyphenolic compounds themselves did not produce significant changes. Three weeks of regression after the 8-week treatment with CCl4 did not lead to a remarkable decrease of endothelium, however, administration of red wine polyphenols during this 3-week period lead to a significant decrease of circulating free endothelial cells in blood. The endothelium protective effect may be one of the factors that contribute to the preventive action of red wine on cardiovascular diseases. Supported by VEGA grants N° 1/9302/02 and 1/0540/03.

CAN RED WINE POLYPHENOLS HAVE PROTECTIVE EFFECT ON LIVER DAMAGE?
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Oxidative stress appears to play a key role in liver cell injury, frequently connected with hepatic steatosis. Polyphenolic compounds with strong reactive radical scavenging activity can influence this process. They have protective effect in various pathological states in different tissues. The presented study evaluates their effect on development of long-term experimental liver injury induced by carbon tetrachloride (CCl4). Wistar rats were divided into 6 groups: control group, a group receiving red wine extract with polyphenols in drinking water (40 mg/kg/day), a group receiving CCl4 subcutaneously (0.5 ml/kg) twice a week, a group receiving both CCl4 and polyphenols, for a period of 10 weeks. Two separate groups receiving CCl4 for 10 weeks were allowed to recover for a 3-week regression period with and without polyphenols administration. CCl4 treatment led to liver steatosis that was not affected by red wine polyphenols. During the 3 weeks of regression a significant reduction in steatosis was found. It is noteworthy that it was significantly more pronounced if polyphenols were given to the animals. NO synthase activity in the liver was dramatically reduced by CCl4 treatment, with significant increase during the regression period. Administration of red wine polyphenols significantly increased the NO synthase activity in all groups, the control group included. It is likely that red wine polyphenolic compounds with reactive radicals scavenging properties participate on reduction of steatosis during the regression period. This finding could be also explained by their effect on reparative processes, making them more effective, where increased NO synthase activity could be an active factor. Supported by VEGA grants N° 1/93 03/02 and 1/05 40/03.
THE EFFECT OF RED WINE POLYPHENOLS ON THE BEHAVIOR PARAMETERS OF NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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Inflammation, immune reaction, ischemia, oxidative stress, viral infections, physical factors, neoplasia and degenerative processes include apoptosis as pathogenetic mechanism. The role of nitric oxide (NO) in apoptosis is controversial. NO is known to induce apoptosis especially in cells of nervous system. NO released from cardiomyocytes under ischemia/reperfusion exerts an antiapoptotic effect, however, NO promotes apoptosis in vascular smooth muscle cells. The function of NO in apoptosis is not clear and more studies are needed to bring light into this problem.

CHARACTERISTICS OF CONDUIT ARTERIES IN NEWBORNS OF NO DEFECTIVE HYPERTENSIVE RATS
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The aim of the study was to characterize the conduit arteries in newborns from nitric oxide (NO) deficient parents. Both parents were administered N-nitro-L-arginine methyl ester (L-NAME) in a dose 40 mg/kg b.w. in drinking water for 4 weeks before mating, and mothers continued during whole pregnancy and breast-feeding. Six newborns of five NO defective hypertensive parents and nine newborns of 3 control parents all at the age of 28 days were taken for the study. Blood pressure (BP) was measured non-invasively on the tail artery using plethysmographic method. The newborns were perfused with a glutaraldehyde fixative under pressure of 120 mmHg. The thoracic aorta (TA) and carotid artery (CA) were processed for electron microscopy. Wall thickness (WT), cross sectional area (CSA), inner diameter (ID) were measured in light microscopy. Volume densities (VD) of smooth muscle cells (SMC) in tunica media of CA were determined in electron microscopy. To characterize the biomechanical features of the vessels, tension in the vessel wall was calculated from measured values. Functional ability of SMC was determined as maximum contraction to noradrenaline by measuring isometric tension of aortic rings in organ bath. BP (mmHg) of experimental newborns was 150±0.2 vs. 104±0.1 in controls. The ratio of body weight to heart weight was 3.9±0.1 vs. 4.5±0.2 in controls, p<0.05 indicated hypertrophy of the heart. Geometry of TA and CA revealed in experimental newborns decreased: WT (µm) (in TA: 50.2±1.5 vs. 63.5±1.3 in controls, in CA: 22.5±0.7 vs. 27.4±0.6 in controls), in CSA (µm²) by 37.62±0.98 % of the whole CSA of tunica media (p<0.01) (i.e. 14.7 ± 0.6 x 10³ µm² of the whole CSA of tunica media), in control carotid artery volume density of SMC was 44.76±1.06 % of tunica media (p<0.01), (i.e. 20.0±1.02 x 10³ µm² of the whole SMC of tunica media). Findings indicate pronounced hypertrophy of SMC in tunica media. A clear-cut increase in wall tension was found in both experimental vessels: in TA by 100 %, in CA by 80 % indicating the increase of mechanical load of the vessel wall. Maximal isometric contraction to noradrenaline was in TA 4.79 mN/mm² vs. 7.19 mN/mm² in control. These findings are in a good consent with hypertrophy of SMC. In conclusion: A high BP, hypertrophy of the heart, decrease of WT in both TA and CA, decrease VD of SMC in CA, increase of wall tension in TA and CA, and decrease SMC contraction ability of TA suggest a strong compromising of cardiovascular system of newborns of NO defective parents. Supported by VEGA 2/3145/23, 2/3166/23 and Slovakofarma, J.S.C., Hlohovec.

GEOMETRY OF THE CORONARY ARTERY OF SHR AND WISTAR RATS DURING ONTOGENIC DEVELOPMENT

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The aim of the study was to evaluate geometry of the septal branch of the ascending coronary artery (RS) of Wistar and SHR during ontogenic development. SHR and Wistar rats ages of 3 weeks (3w), 9 weeks (9w), 17 weeks (17w), 52 weeks (52w) were studied. The rats were perfused with glutaraldehyde fixative under pressure 90 mmHg (3w), and 120 mmHg (the rest of groups). Upper part of RS was excised and processed according to standard electron microscopy procedure. Wall thickness (WT) and inner diameter (ID) were measured and cross sectional area (CSA) and wall thickness/inner diameter ratio (WD) were...
calculated. BP of 3w old Wistar rats (83±2 mmHg) did not differ from the age matched SHR (84±1 mmHg). In 9w old Wistar rats BP was 106±1 mmHg vs. 154±1 mmHg in SHR, p<0.01; in 17w old Wistar rats BP was 114±1 mmHg vs. 214±7 mmHg, p<0.01; and in 52w Wistar rats it was 115±5 mmHg vs. 190±3 mmHg, p<0.01. HW/BW ratio was increased in all SHR groups (p<0.01) and indicated myoccardial hypertrophy. (mm) of SHR was increased in all ontogenic period: 3w old rats 9.6±0.25 vs. 11.1±0.59, p<0.05; 9w old rats 10.6±0.88 vs. 13.8±0.59, p<0.01; 17w old rats 9.33±0.67 vs. 20.1±1.25, p<0.01; 52w old rats 11.07±0.51 vs. 21.36±1.34, p<0.01. Changes in ID (µm) was observed only in groups of 52w (266±13 in control vs. 414±14 in SHR, p<0.01). No differences in this respect were found among the groups of 3w 182±6 vs. 163±11; 9w 220±11 vs. 212±5; and 17w 250±12 vs. 321±26. CSA (x10² µm²) of 3w rats did not change between groups (5.8±2.4 vs. 6.1±0.6). The increase in CSA was observed between the groups of 9w (7.6±0.6 vs. 9.8±0.4, p<0.01); 17w (7.5±0.8 vs. 21.7±2.4, p<0.01); 52w (9.7±1 vs. 29.5±2.4, p<0.01). WD (x10²) was increased in all SHR groups in comparison to age matched Wistar rats: 3w 7.0±0.48 vs. 15.3±0.1. CSA of SHR was 6.7±5.6 vs. 7.9±3.4 in SHR, p<0.05. CSA (x10² µm²) of 3w rats did not change between groups (6.2±1.3 vs. 6.1±0.8). The increase in CSA was observed between the groups of 9w (11.5±3 mmHg vs. 190±3 mmHg, p<0.01). However, there is a little information on the effect of PB on cardiovascular system. Experiments were performed to show the possible effect of nitric oxide is speculated. The effects of PB on cardiovascular system. Experiments were performed to show the possible effect of nitric oxide is speculated. The effects of PB on cardiovascular system. Experiments were performed to show the possible effect of nitric oxide is speculated.
pattern of fat distribution was present even in lean HBP subjects, RAC seems to be a better measure of prevalence of central adiposity as compared to WHR. Conclusion: Whereas during childhood and adolescence also accelerated growth and maturation contribute to elevated BP, in adults the most pronounced association was with prevalence of central fat distribution.

DETECTION OF R243X AND Y414C MUTATIONS IN THE PHENYLALANINE HYDROXYLASE (PAH) GENE. RELATION OF PAH AND NO SYNTHASE

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Phenylketonuria (PKU) is a relatively common monogenic autosomal recessive disorder. This inborn error of amino acid metabolism is caused by mutations in the gene (localized on the 12th human chromosome, position 12q22-24.1) encoding liver-specific enzyme phenylalanine hydroxylase (PAH). In the Slovak PKU patients the identification of eight mutations was carried out by the methods of direct DNA diagnosis using either naturally occurring restriction sites (R408W, R261Q, R252W, IVS10nt546, G272X and R408Q), or amplification created restriction sites (IVS12nt1 and R158Q).

The study presents results of the direct detection of mutations R243X and Y414C in the PAN gene detected on 47 PKU patients of Slovakia.

The screening for mutations R243X and Y414C was performed using the amplification created restriction site (ACRS) technique. Firstly, the exons 7 and 12 of the PAH gene were amplified. Secondly, the amplification products (102bp and 147bp respectively)were digested using restriction enzymes Mspl (R243X) and Rsal (Y414C). Then restriction fragments were separated in 1.5% agarose gel. The restriction sites were devised so as to be abolished in presence of mutations altering these sites, in both cases. Of the 94 mutant alleles analyzed, three were found to carry Y414C mutation and none of them was detected to carry R243X mutation.

The results of this study were combined with the data of screening for 8 PAH mutations from the Slovak population. From the total number of 19 PAH mutations which were sought, 7 were identified in our PKU patients, which accounted for 70.7 % of mutant PAH alleles in Slovakia (133/188). The most common molecular defect was the mutation R408W in 47.3% of all PAH alleles (89/188). IVS12nt1 accounted for 6.9 % of PAH alleles and each of two other mutations (R158Q, R261Q) accounted for 4.8 % of PAH alleles. Rarely present were mutations R252W (3.7 %), IVS10nt546 (1.6 %) and Y414C (1.6 %). Mutations G272X, R408Q and R243X, that have been described in the neighbouring countries (Czech Republic, Poland, Hungary) and in other European countries, have not yet been detected in Slovakia. However, seven of the previously reported mutations were found to account for 70.7 % of the PAH mutations in Slovak patients of Caucasian origin, 29.3 % of the mutations are still unknown.

STRESS RESPONSIVENESS IN OXYTOCIN KNOCKOUT MICE

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Studies investigated the role of oxytocin (OT) in blood pressure regulation and stress reactivity in OT knockout (OTKO) mice. Male OTKO (OT-/-) and control (OT+/+) mice with chronic arterial catheters were exposed to 7 days of shaker stress (2-min periods, 45 times/day). The immediate MAP and HR responses were analyzed in the dark and light periods (19.00h and 08.00h). In the light, stress-induced MAP increases were seen on stress days 1, 3, 7 and 1 day post-stress recovery in OTKO and on stress days 1 and 3 in controls. In the dark, stress induced pressor responses were seen only in OTKO (stress days 1 and 3), but no in controls. There were no genotype-related differences in HR responses. Plasma corticosterone was measured before stress exposure and 30 min after the last shaking session on day 7. OTKO mice showed lower responses than controls (increase 298% vs. 411%, p<0.05). In conclusion, deletion of the OT gene altered endocrine and pressor stress responsiveness in mice. The data provides evidence for an antistress effect of endogenous OT in blood pressure regulation. Supported by the US DoD contract No. DAMD17-00-C-0020.