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The Role of Nitric Oxide in Cardiovascular System

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POLYPHENOLS: PROTECTION OF NEUROVASCULAR UNIT IN STROKE AND INHIBITION OF IN—STENT—NEOINTIMAL GROWTH R Andriantsitohaina[1], Y Curin[1,2], MF Ritz[2], R Gérald[3], A Alvès[4], A Mendelowitsch[2], M Elbaz[5], *[1]UMR CNRS 7081, Illkirch, France, [2]Neurosurgery lab, Basel University Hospital, Basel, Switzerland, [3] Cardiology Hautepierre, Strasbourg, France, [4]Biomatech, Pathology Institute, Chasse-sur-rhône, France [5]Cardiology Rangueil, Toulouse University Hospital, France*

Epidemiological studies have suggested that diet rich in polyphenols can reduce the risk of cardiovascular diseases. Indeed, these compounds possess a number of biological effects including anti-aggregatory platelet activity, antioxidant and free radical scavenging properties. Another therapeutically relevant effect of polyphenols may be their ability to interact with the generation of nitric oxide from vascular endothelium that leads not only to vasodilatation but also to the expression of genes protective of the cardiovascular system. Also, polyphenols contribute to the preservation of the integrity of cells belonging to the neurovascular unit mainly the endothelium by acting on the signaling cascades implicated in endothelial apoptosis, proliferation and migration. All these effects of polyphenols might interfere with atherosclerotic plaque development and stability, vascular thrombosis and occlusion. Two properties of chronic treatment with polyphenols from red wine, Provinols™, will be discussed.
Firstly, the ability of Provinols™ by protecting vascular, neurons and other brain cells against cerebral ischemia in rat submitted to a middle cerebral artery occlusion as a relevant experimental model of stroke has been assessed. The ability of Provinols™ to modify brain energy metabolism, oxidative stress and cerebral blood flow during ischemia and reperfusion was tested. The data suggest that chronic treatment with polyphenols inhibits ischemia-induced excitotoxicity by abolishing the burst release of aspartate and glutamate and by improving cerebral blood flow. Thus, Provinols™ may represent a potential neuroprotection against stroke.
Secondly, orally effective drugs in inhibiting neointimal growth after experimental angioplasty is still a big challenge to prevent or reduce restenosis after stenting. Our study was designed to investigate the potential of Provinols™ to decrease neointimal hyperplasia after angioplasty in a hypercholesterolemic-fed rabbit model. The results show that oral administration of Provinols™ reduces in-stent neointimal growth, lipid deposition in association with its anti-inflammatory property in iliac arteries from hypercholesterolemic rabbits. Moreover, they provide an experimental basis for the beneficial effects of plant-derived polyphenols for the prevention of restenosis associated with stent placement.

RELATIONSHIP BETWEEN NITRIC OXIDE AND APOPTOSIS P. Babál, *Department of Pathology, Comenius University, Bratislava, Slovak Republic*

Apoptosis is characterized by an organized collaps of the cell with formation of bulging saccular processes, a general retraction of the cell, condensation of nuclear chromatin, fragmentation of DNA and phagocytosis of cell fragments by macrophages. Different from necrosis, this programmed cel death does not lead to cell decomposition and to an inflammatory reaction. Apoptosis participates on physiological elimination of cells under physiological conditions, takes place in fetal and embryonal development, spontaneously occurs in tumor cells, can be also evoked by cell-mediated immunity or by various substances, toxins. The external activation signals mediated by TNF receptors family through binding of adaptor proteins in the cytoplasm. The internal activation path involves mitochondria that release cytochrom c as a reaction to various noxious stimuli, damage of DNA included. Sphingomyelin signaling path with ceramide formation is one of the basic factors in apoptosis activation mechanisms in stress of the cell. Process of apoptosis is highly regulated by genes and their products on the cell membrane, in cytosole and in mitochondria. One of the key proteins is the membrane TNF receptor (CD95, Fas) with its intracellular death domain. Transcripts of the bcl-2 gene family support or inhibit apopotosis. Cystein proteases, caspases, represent the apoptosis effector system that inactivate proteins of the DNA reparative enzymes, the cytoskeletal proteins, modulate oncoproteins and DNA-ases. Inflammation, immune reaction, ischemia, oxidative stress, viral infections, physical damage, neoplasia and degenerative processes include apoptosis as pathogenetic mechanism. The role of NO in apoptosis is controversial. NO can inhibit cell death through function as a radical species scavenger, induction of protective proteins, S-nitrosylation of caspases, Bcl-2 cleavage and cytochrome c release inhibition, resulting in inhibition of apoptosis. NO can also induce cell death through p53 accumulation and expression of Bcl-2 family proteins. NO can shift apoptotic response into necrotic death. Signaling pathways of NO leading to or inhibiting apoptosis remain still poorly understood. The function of NO in apoptosis is ambiguous and more studies are needed to comprehend its biological functions.

THE ROLE OF INTRACELLULAR PROTEIN KINASE PATHWAYS IN THE EFFECTS OF CHRONIC NOS INHIBITION IN THE RAT HEART M. Barancik, P. Simoncikova, M. Strniskova, [1]O. Pechanova, T. Ravingerova. *Institute for Heart Research,[1] Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Slovak Republic*

Nitric oxide (NO) has been implicated in the mechanisms of cardiac adaptation to ischemic stress but the impact of chronic NO deficiency (NOD) on the mechanisms of ischemic tolerance has not been sufficiently elucidated so far. We looked for 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 in rat hearts. NOD was induced by L-NAME (40/mg daily, 4 weeks). Isolated hearts from control rats or rats with chronic NOD were Langendorff-perfused and subjected to test index ischemia (II) induced by 25 min global ischemia and 35 min reperfusion. We found that hearts from rats with chronic NOD showed better recovery of contractile function after II. The development of NOD was connected with decreased activation of extracellular-signal regulated protein kinases (ERK). During IR we found activation of ERK-2 in comparison to basal conditions and this activation was observable also in NOD rats. The study of p38-MAPK, a kinase that belongs to the group of "stress" kinases, did not show significant changes in activation of this kinase with development of NOD. L-NAME treatment induced changes also in expression of iNOS and inhibited the phosphorylation of eNOS. Moreover, the activities of tissue matrix metalloproteinase 2 (MMP-2) were significantly decreased in hearts of NOD rats. The effects of NOD on ERK pathway and the changes in ERK activation during IR suggest the involvement of this signaling pathway in adaptive responses of myocardium. The results point also to the possible relationship between ERK pathway activation and activation of eNOS and /or MMP-2.
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ENDOTHELIAL DYSFUNCTION IN THE POST-ISCHEMIC HEART – ITS MECHANISM AND THE MECHANISM OF PROTECTION BY ISCHEMIC PRECONDITIONING A. Beresewicz, M. Maczewski, M. Duda, *Medical Centre of Postgraduate Education, Warsaw, Poland*

Ischemia/reperfusion (IR) results in coronary endothelial dysfunction and subsequent deleterious neutrophil accumulation in the post-ischemic myocardium, the effects preventable by superoxide dismutase (SOD), endothelin ET_A receptor blockade, and ischemic preconditioning (IPC). In this context, we have tested the hypothesis that: (i) endothelin-induced process is responsible for the cardiac superoxide (O_2^-) overproduction that mediates the post-ischemic endothelial dysfunction; (ii) a reaction product between O_2^- and nitric oxide (NO) rather than O_2^- per se mediates the endothelial dysfunction; (iii) IPC protects the endothelium by preventing post-ischemic cardiac ET-1, and subsequent O_2^- (or NO) formation, and (iv) that the opening of the mitochondrial ATP-dependent potassium channel (mK_{ATP}) plays a role in the mechanism of IPC. Langendorff-perfused guinea-pig hearts were subjected either to 30 min global ischemia/30 min reperfusion (IR) or were preconditioned prior to IR with three cycles of either 5 min ischemia/5 min reperfusion or 5 min infusion/5 min wash-out of mK_{ATP} opener, diazoxide (0.5 μM). Coronary flow responses to acetylcholine (ACh) and nitroprusside (SNP) were used as measures of endothelium-dependent and endothelium-independent vascular function, respectively. Myocardial outflow of ET-1, O_2^- and NO were followed during reperfusion. IR impaired the ACh response by approximately 60% and caused a burst of cardiac ET-1, O_2^- and NO outflow. All these changes (with the exception of NO burst) were prevented by IPC and diazoxide preconditioning, and the effects of preconditioning were prevented by a blocker of mK_{ATP}, hydroxydecanoate, given only prior to IR. Diazoxide given after 30-min ischemia increased the O_2^- burst and was not protective. The impairment of ACh response and the O_2^- burst were prevented by the endothelin ET_A/ET_B receptor antagonist, tezosentan, and SOD. NO synthase inhibitor, L-NMMA, inhibited the burst of NO, but not of O_2^-, and afforded protection of the ACh response in IR hearts. NO scavenger, oxyhemoglobin, afforded similar endothelial protection. The results suggest that in guinea-pig heart: (i) it is post-ischemic release of ET-1 that mediates O_2^- burst and related endothelial dysfunction; (ii) a reaction product between $.O_2^-$ and NO (e.g., peroxynitrite) rather than $.O_2^-$, per se, mediates the post-ischemic endothelial dysfunction; (iii) the mK_{ATP} opening serves as a trigger, but not effector, of IPC- and diazoxide-induced endothelial protection, and (iv) the mK_{ATP} opening protects the endothelium in the mechanism that involves the attenuation of ET-1, O_2^-, and perhaps peroxynitrite production at reperfusion.

ACETYLCHOLINESTERASE INHIBITION AFFECTS CARDIO-VASCULAR STRUCTURE IN MICE. I. Bernatova,[1,2] P. Babal,[3] R.D. Grubbs,[1] M. Morris[1] *[1]Department of Pharmacology and Toxicology, Wright State University School of Medicine, Dayton, Ohio; [2]Institute of Normal and Pathological Physiology Slovak Academy of Sciences, Bratislava, Slovakia; [3]Institute of Pathological Anatomy, Medical Faculty, Comenius University, Bratislava, Slovakia*

Pyridostigmine bromide (PB), a quaternary ammonium compound, reversibly inhibits acetylcholinesterase (AChE), an enzyme involved in the ACh metabolism and thus in regulation of autonomic function. It is used clinically to treat myasthenia gravis and as a prophylactic against organophosphate poisoning. However the effects of PB in cardiovascular system are not adequately understood. In this study we investigated the effect of PB, shaker stress and their interaction on cardiac and aortic structure, including apoptotic markers (Bax and Bcl-2) in mice. In order to determine whether any structural changes were related to cardiovascular function, we also determined the effect of PB and stress on mean arterial pressure (MAP), heart rate (HR) and stress-induced cardiovascular reactivity. Experiments were performed in adult male C57BL/6J mice. Mice were studied under the following conditions: Controls (saline-treated), PB (10 mg/kg/day, 7 days), shaker stress (7 days, 45 sessions/day, 2 min/session) and PB+Stress combination. PB and saline were administered using Alzet® osmotic minipumps. AChE activity was reduced in all PB-treated animals by about 46% ($p<0.001$ *vs.* control). PB caused no changes in 24-h MAP or HR *vs.* control. Stress alone increased 24-h MAP on day 1 and 24-h HR on day 7 of treatment in both Stress and PB+Stress groups. In PB+Stress group, PB accentuated stress induced pressor reactivity during the dark period. Relative heart weight was slightly reduced in the PB group while no alterations were observed in PB+Stress group. There were no changes in cardiac fibrosis in any group investigated as compared with the Control group. There was a four-fold increase in Bax/Bcl-2 ratio in the heart of both PB and PB+Stress groups, suggesting an increase in apoptosis. In contrast, there was an attenuation of apoptosis in the aortic endothelium in PB and PB+Stress treated mice. However, a significant reduction in the aortic wall thickness/diameter ratio was found only in PB-treated mice. On balance then, the results suggest adverse influence of chronic PB treatment on growth of the heart and aorta of mice that was eliminated by simultaneous stress exposure. Data also suggest that PB may affect apoptosis in the cardiovascular system. Observed effects of PB in the heart and aorta were no associated with changes in MAP and HR. Study was supported by the US Department of Defense contract No. DAMD17-00-C-0020 and partially by VEGA grant No. 2/4156/04.

AN IN SITU EVIDENCE FOR AUTOCRINE FUNCTION OF NITRIC OXIDE
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NO originates via the oxidative L-arginine pathway catalyzed by a NO-synthase (NOS) family constituted by three distinct NOS isoforms (NOS1, NOS2 and NOS3). With the advent of powerful immunohistochemical techniques, it turned out, that NOS1-3 are not unique for specific cell types (1;2) and have a wide tissue distribution. The conventional classification of NOSs into neuronal, endothelial and inducible NOS reflects only characteristics of the cells, in which the enzymes were first described, and may cause confusion. The classification of NOSs as “constitutive” or “inducible” has also turned out to be unreliable because each of the isoforms may be regulated dynamically (3;4). Finally, mathematical modeling has presently revealed that the spatial-temporal gradient of the NO diffusion rate in biological media was largely overestimated (5).

To address this issue, we examined *in situ* localization of NOSs in blood vessels of the elastic and muscular types and found all three NOS isoforms co-expressed in the smooth muscle depending on the blood vessel type. The expression pattern of NOS1-3 in vascular smooth muscle cells showed striking parallels with enzymes of NO signaling cascade (arginase, phosphodiesterase and soluble guanylyl cyclase), which is indicative of an autocrine rather than paracrine fashion of NO signaling in the vasculature (6;7). Our findings challenge the commonly accepted view, that the expression of NOS is restricted to vascular endothelial cells and imply that the constitutive local NOS expression in the smooth muscle may modulate vascular functions in an endothelium-independent manner. Co-expression of NOS with the enzymes engaged in the NO-signaling was also found in the myocardial and skeletal muscles, as well as in the kidney, pancreas and liver. Finally, our ultrastructural studies with immunogold labeling gave evidence for an autocrine fashion of NO signaling not only at the tissue level but also at the level of individual subcellular compartments.

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GEOMETRY OF CORONARY AND BASILAR ARTERIES IN SHR DURING ONTOGENIC DEVELOPMENT M. Cebová, F. Kristek *Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia.*

The aim of the study was to evaluate the geometry of the septal branch of the left descending coronary artery (RS) and the basilar artery (AB) of Wistar rats and spontaneously hypertensive rats (SHR) during the four periods of ontogenic development. Four groups of Wistar rats of the age: 3 weeks (3w), 9 weeks (9w), 17 weeks (17w) and 52 weeks (52w), and four groups of the age matched SHR were taken for the study. Blood pressure (BP) was measured non-invasively on the tail artery using the plethysmographic method. Under anaesthesia the rats were sacrificed and perfused with a glutaraldehyde fixative under the pressure of 90 mm Hg (3w) and 120 mm Hg (the rest of groups). Hearts were weight and heart weight/body weight ratios were calculated. RS and AB were excised and processed according to standard electron microscopic procedure. Wall thickness (WT) and inner diameter (ID) were measured on semithin sections using light microscopy, and cross sectional area (CSA) and WT/ID ratio (WD) was calculated. BP of 3w old SHR did not differ from the age matched control Wistar rats. In comparison to age matched controls increase of BP was observed in 9w (by 44 %), in 17w (by 88 %), and in 52w (by 66 %) old SHR. The value of heart weight weight/body weight ratio was, in comparison to age matched controls, higher in all SHR groups and indicated cardiac hypertrophy. In both control arteries only CSA and ID continually increased during the ontogeny, WT and WD of did not changed. In both SHR arteries WT, CSA, ID in RS, and WD in AB during the ontogeny continually increased. ID of AB and WD of RS did not changed (except of 52w where was in comparison to 17w decreased).
Comparison of the arteries of age matched SHR and control groups revealed that: in 3w old SHR only WD was increased, in 9w, 17w, and 52w WT, CSA, WD was increased in both arteries. Summarising: In comparison to Wistar rats we have already found in prehypertensive period of SHR cardiac hypertrophy and remodelled wall of both coronary and basilar artery. The alterations in geometry of the arteries increased remarkably with differences in blood pressure.
The study was supported by VEGA grant 2/3145/23, Slovakia.

NITRIC OXIDE DRIVEN APOPTOSIS IN RETINAL DEGENERATIONS FROM CELL LINES TO ANIMAL MODELS
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The loss of photoreceptor and related cells via apoptosis is seen in several degenerative conditions of the retina such as retinitis pigmentosa, macular degeneration and glaucoma and a role for nitric oxide and oxidative stress in this cell loss has been strongly suggested. In this study we describe the molecular events occurring after the treatment of the photoreceptor cell line 661W cells and cultured retinal explants with agents that induce oxidative stress. The generation of reactive oxygen and nitrogen species were detected by flow cytometric methods. Apoptosis was detected by propidium iodide staining, DNA ladder formation and annexin V staining. We show that increased cytosolic levels of calcium occur during photoreceptor apoptosis and this leads to the activation of calpains. As calpain activation is occurring there is a corresponding loss of the endogenous calpain inhibitor calpastatin, this being detected by western blotting. We also demonstrate that caspase activation occurs following treatment with SNP. However, treatment with the pan caspase inhibitor zVAD-fmk (zVal-Ala-Asp-fluoromethylketone), while it blocks caspase activity, does not prevent apoptosis. On the other hand we demonstrate that CR-6 (3,4-dihydro-6-hydroxy-7-methoxy-2,2-dimethyl-(2H)-benzopyran) a vitamin E analogue acts as a scavenger of nitric oxide and reduces 661W photoreceptor apoptosis induced by SNP by preventing the activation of a pathway in which calpains have a key role. In summary this is the first report that both caspases and calpains are involved in 661W photoreceptor apoptosis and that calapin activation and apoptosis can be prevented with the reactive nitric oxide scavenger CR-6. It also suggests a possible therapeutic avenue for the prevention of cell loss during retinal degeneration.

EFFECT OF STRESS ON VASCULAR REACTIVITY IN NORMOTENSIVE AND HYPERTENSIVE RATS

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Genetic predisposition and stress are known risk factors in the etiology of hypertension. However, the mechanism of stress-induced hypertension is not satisfactorily understood.

The aim of the study was to investigate the effect of psychosocial stress produced by crowding on vascular reactivity in normotensive (Wistar) and spontaneously hypertensive (SHR) rats and to evaluate genotype- and stress-related differences between normotensive and hypertensive rats. Adult, 12-weeks-old, males of both genotypes were exposed to crowding for 8 weeks (200 cm^2/rat, 5 rats/cage) while controls were kept 4 rats/cage, 480 cm^2/rat. After eight weeks of crowding, blood pressure (BP) was increased significantly in SHR (195±3 mm Hg *vs.* 184±3 in control SHR, $p<0.05$). BP of Wistar rats was not affected by stress. Vascular function was investigated in rings of the femoral artery with functional endothelium using Mulvany's myograph.. Phenylephrine-induced constriction (10^{-4} mol/l) was elevated in SHR by about 49% *vs.* Wistar ($p<0.05$, main effect of genotype) and no effect of stress was observed. Acetylcholine-induced relaxation was comparable in both genotypes with the average values 58±7 and 65±5% in Wistar and SHR, respectively, and stress failed to affect significantly these values. However, 20-min pre-incubation of vascular rings with low dose L-NAME (10^{-6} mol/l) resulted in reduced relaxation only in stress-exposed SHR (-13%, $p<0.03$). No significant effect of low-dose L-NAME pre-incubation was observed in Wistar rats. NA-induced constriction was elevated in SHR by about 36% *vs.* Wistar ($p<0.005$, main effect of genotype) and stress increased it by about 65% *vs.* control ($p<0.001$, main effect of stress). The analysis of interaction of stress and genotype revealed the elevation of NA-induced constriction only in stress-exposed SHR (by about 90% vs. control SHR, $p<0.001$), the effect was not observed in Wistar rats. Serotonin did not induce any genotype- or stress-related alterations in constriction of the femoral artery.

Data provide the evidence that 8 weeks of social stress led to elevation of BP only in hypertensive rats. This was associated with elevation of NA-induced constriction, suggesting the role of sympathetic nerve system. However, these effects of stress were no observed in normotensive rats.

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CHANGES IN VASCULAR REACTIVITY AND GEOMETRY OF ILIAC ARTERY IN SPONTANEOUSLY HYPERTENSIVE AND HYPERTRIGLYCERIDEMIC RATS

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We characterized the vascular reactivity and structural changes of iliac artery (IA) in two experimental models - hereditary hypertriglyceridemic (HTG) rats characterized by insulin resistance, hypertriglyceridemia and elevated blood pressure which mimics the situation in "metabolic syndrome" and spontaneously hypertensive rats (SHR) which represents a model of essential hypertension. Male Wistar rats (control), SHR, and HTG rats 17 weeks old were used. Systolic blood pressure (BP) was measured on the tail artery using plethysmographic method. Animals were anesthetized, for functional study the right IA were ligated and excised. Relaxant responses were studied after application of acetylcholine (ACh, 10^{-8} - 10^{-5} mol/l)) on phenylephrine (10^{-5} mol/l)-precontracted isolated ring of IA. Contractile responses were induced by noradrenaline (NA, 10^{-9} - 3.10^{-5} mol/l). For morphological study the rats were perfused with fixative and the left IA were processed for electron microscopy. The geometry of IA was measured using light microscopy. BP was significantly increased in SHR group by 100% and HTG group by 62 % and it was accompanied by increased heart weight to body weight ratio signalizing cardiac hypertrophy. Endothelium-dependent relaxation to ACh was significantly attenuated in both experimental groups (22,2±5,54% in SHR and 29,71±6,43% in HTG vs. 74,87±8,43% in controls, $p<0,05$). There was found a similar enhancement of maximum contraction to adrenergic stimuli in both experimental groups. Sensitivity to NA was increased in SHR group compared to HTG group. Morphometry in both experimental groups showed significantly increased wall thickness (WT), wall cross-sectional area (CSA), wall thickness/inner diameter ratio and decreased inner diameter. WT and CSA in SHR group (54,12±1,99 μm and 122 804±6298 μm^2, respectively) were significantly increased compared to HTG group (47,74±1,73 μm and 97811±2343 μm^2, $p<0,05$, respectively). Independent of etiology, both types of hypertension revealed impaired endothelial function accompanied by structural alterations of IA. The value of BP correlated with arterial wall hypertrophy and increased smooth muscle sensitivity to contractile stimuli.

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NITRIC OXIDE IN ONTOGENESIS - CONSEQUENCES OF COMPROMISED PRODUCTION.

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With the aim to recognize the role of nitric oxide (NO) in cardiovascular system in ontogenesis, following issues were studied: 1) Nitric oxide relaxation in conduit arteries of mongrel canine fetuses and newborns 1-6 week old, and

2) Cardiovascular system - structure and function - in offspring of rat NO defective hypertensive parents. Rings of canine thoracic aorta and carotid a. of fetuses and newborns in vitro responded to Ach by isometric relaxation which was dose-dependent and significantly greater than responses of vessels from adult animals. The question was posed whether NO is responsible and triggers the relaxation of smooth muscle cells. The inhibition of NO synthase was induced in newborns from the first week and lasted 5 weeks (L-NAME 50 mg/kg b.w/day s.c.). Blood pressure in the newborns was 168±5 mmHg vs 95±14 mmHg in controls. The relaxation of aorta and carotid a. ring after NO synthase inhibition was attenuated partly, proving that NO is operating in conduit artery wall already in this early period. Since, however, a serious range of isometric relaxation of the vessels to Ach maintains even after NO synthase inhibition, other relaxing mechanisms are supposed to operate even in the early ontogenetic period. The rat offspring stemmed from parents with high BP, induced by inhibition of NO synthase, lasting 5 weeks in males, and 8-9 weeks in females (breast feeding inclusively). The newborns 3-4 weeks old have high BP (150±2 mmHg vs. 104±2 mmHg in controls). NO synthase activity in the myocardium was lower in comparison to age matched controls. Ornithine decarboxylase activity in the myocardium and thoracic aorta was higher, indicating a positive growth-trophic effect. Contrary to the above three findings, as well as to general paradigma, a lower Heart weight/Body weight ratio was found indicating a hypotrophy of the heart. The controversial finding was further accentuated by the decrease of aorta and carotid a. wall thickness and wall thickness/inner diameter ratio, indicating the hypothrophy of the conduit arteries too. The rate of Ach relaxation of conduit arteries from NO defective hypertensive offspring did not differ from controls. The findings indicate a serious impairement of CV system, the relaxing ability of conduit arteries seems to be preserved. Supported by VEGA grants 2/3145/25 and 2/3166/25.

THE EFFECT OF RED WINE POLYPHENOLS ON THE CARDIOVASCULAR DAMAGE INDUCED BY LONG-TERM TETRACHLORMETHAN TREATMENT

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Carbon tetrachloride intoxication is a model of system toxicity caused by free radicals and reactive aldehyde formation. Oxidative stress appears to play a key role in cardiovascular injury. The enzymes responsible for the metabolic activation of carbon tetrachloride were detected also in the heart and vascular wall muscle. Polyphenolic compounds with strong reactive radical scavenging activity and the ability to increase nitric oxide synthase activity can influence this process. Their protective effect in various pathological states in other tissues has been shown. The presented study evaluates their effect on the development of experimental cardiovascular injury induced by carbon tetrachloride. Male Wistar rats were divided into 6 groups: control group, a group receiving 12 weeks CCl_4 subcutaneously (0,5ml/kg) two times a week, a group that after that was allowed to recover for a 3-week regression period and the same group that received in addition red wine extract with polyphenols in drinking water (30mg/kg/day). NO synthase activity in the heart was dramatically reduced by CCl_4 treatment, with significant increase when polyphenols were administrated. CCl_4 administration caused endothelial injury in the arteries, followed by endothelial dystrophy with vacuolar degeneration and detachment of endothelium. The arterial media showed enlargement of intercellular spaces and cystic degeneration. Parallel administration of red wine polyphenols showed protective effect on vascular damage in all groups. On the other hand, polyphenols significantly increased the wall thickness/diameter index in all groups and the cross sectional area of aorta in the regression group. It is likely that red wine polyphenolic compounds participate on reduction of toxic vascular injury caused by carbon tetrachloride. They can positively influence the reparative processes in the vascular wall.

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THE EFFECT OF DIURETICS ON THE DEVELOPMENT OF SPONTANEOUS HYPERTENSION L. Jendeková[1], S. Kojšová[1], Z. Csizmadiová[1,2], I. Vranka[3], R. Janíková[3], O. Pecháňová[1] *[1]Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, [2]Faculty of Natural Sciences, Comenius University, [3]Medical School, Comenius University, Bratislava, Slovak Republic.*

Diuretics are commonly used drugs with hypotensive efficacy through a combined vasodilator and diuretic effect. In this experiment we compare the preventive effects of thiazide diuretic (hydrochlorothiazide) and non-thiazide indoline (indapamide) on blood pressure development in SHR. Young 6-week-old male SHR were treated with hydrochlorothiazide (HCT) in the dose 10 mg/kg/day and indapamide in the dose 1 mg/kg/day for six weeks. Systolic blood pressure (SBP) was measured by tail-cuff plethysmography. Total nitric oxide synthase (NOS) activity was determined by measuring the formation of L-[^{3}H] citrulline from L-[^{3}H] arginine in the left ventricle, aorta, kidney and cerebellum. Conjugated diene (CD) concentrations were detected in the left ventricle and kidney. Chronic HCT and indapamide treatment partially attenuated SBP rise in young SHR (154±3 and 157±4 mmHg, respectively, vs. 171±1 mmHg in untreated animals). Indapamide significantly increased NOS activity in the aorta, whereas HCT treatment showed only the increased trend of NOS activity in this tissue. Both indapamide and HCT failed to modify NOS activity in other tissues. Indapamide, in contrast to HCT, attenuated concentration of reactive oxygen species measured as decreased CD concentration in the kidney. Our study demonstrated that indapamide and HCT decreased systolic blood pressure similarly, but only indapamide increased NOS activity in the aorta and decreased CD concentration in the kidney.
In conclusion, these results suggested that besides the diuretic effect, indapamide is able to increase NOS activity and attenuate reactive oxygen species which could potentially contribute to its antihypertensive properties.
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THE EFFECT OF INDAPAMIDE AND CAPTOPRIL ON BLOOD PRESSURE, NITRIC OXIDE GENERATION AND OXIDANT STATUS IN SPONTANEOUSLY HYPERTENSIVE RATS S. Kojšová[1], L. Jendeková[1], Z. Csizmadiová[1,2], I. Vranka[3], R. Janíková[3], O. Pecháňová[1] *[1]Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, [2]Faculty of Natural Sciences, Comenius University, [3]Medical School, Comenius University, Bratislava, Slovak Republic*

The purpose of this study was to compare the effects of different antihypertensive agents, non-thiazide diuretic (indapamide) and angiotensin-converting enzyme inhibitor (captopril) on the development of spontaneous hypertension. The combine effect of these agents was analyzed as well. Six-week-old male SHR were treated with indapamide (1 mg/kg/day), captopril (10 mg/kg/day) and with indapamide+captopril combination. Systolic blood pressure was measured by tail-cuff plethysmography. After 6-week treatment nitric oxide synthase (NOS) activity was determined by measuring the formation of L-[^{3}H] citrulline from L-[^{3}H] arginine in the left ventricle, aorta, kidney and cerebellum and concentration of conjugated dienes (CD) were detected in the left ventricle and kidney. Indapamide (I), captopril (C) and indapamide+captopril combination (I+C) significantly decreased systolic blood pressure in young SHR (I: 157±4, C: 137±2, I+C: 121±1 mmHg) in comparison with untreated animals (171±1 mmHg). Indapamide, in contrast to captopril, increased NOS activity in the aorta and attenuated concentration of reactive oxygen species measured as decreased CD concentration in the kidney. Indapamide+captopril combination increased NOS activity in the aorta and decreased CD concentration in the kidney as well. There were no changes in the NOS activity and CD concentrations in other tissues investigated.
In conclusion, indapamide treatment along with ACE inhibitor captopril had the additive effect on the prevention of blood pressure increase in young SHR. On the other hand, this combination increased NOS activity in the aorta similarly as indapamide alone. Our results suggested that indapamide is responsible for NOS activity increase after I+C combination treatment. This effect of indapamide may contribute to its vasorelaxant and antihypertensive properties.
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EFFECT OF STRESS ON PRESSOR RESPONSE AND LOCOMOTOR ACTIVITY IN RATS WITH VARIOUS FAMILY HISTORY OF HYPERTENSION J. Kopincová[1,3], Z. Csizmadiová[1,3], M. Dubovicky[2], I. Bernátová[1] *[1]Institute of Normal and Pathological Physiology, [2]Institute of Experimental Pharmacology, Slovak Academy of Sciences, [3]Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic.*

Genetic predisposition and psychosocial stress are two main risk factors in etiology of cardiovascular diseases, manifesting themselves also in behavioral profiles. The aim of this study was to investigate and compare effects of psychosocial stress on three different genotypes of rats by monitoring their blood pressure (BP) and locomotor activity. Male 12-week-old normotensive (Wistar) rats, spontaneously hypertensive rats (SHR) and borderline hypertensive rats (BHR, offspring of spontaneously hypertensive dams and Wistar sires) were exposed to 8-week crowding stress (5 rats/cage, 200 cm^2/rat). Control Wistar, SHR and BHR rats were kept 4 rats/cage (480 cm^2/rat). BP was measured in conscious animals by tail-cuff plethysmography once before the stress exposure and four times during the experiment, in the first, the third, the sixth and the eighth week. At the same time, open field tests were done. Horizontal and vertical activity and grooming were monitored in 6-min sessions. Basal BP of Wistar, BHR and SHR rats were 113±1, 137±2 and 185±2 mm Hg, respectively ($p<0.001$ between genotypes). Stress increased BP in BHR and SHR (approximately by 11 and 5 mm Hg, respectively, $p<0.05$ *vs.* control) and had no effect in Wistar. Basal levels of locomotor activity and grooming were significantly different between all genotypes. The lowest levels of locomotor activity were observed in BHR while the highest levels were observed in SHR. Stress exposure resulted in increased horizontal square crossing only in BHR. Vertical activity was not affected by stress in any genotype. On the other hand, grooming was increased significantly in stressed SHR. In conclusion, rats with family history of hypertension showed higher pressor response to social stress than normotensive rats, associated with alteration in horizontal activity and grooming.
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EFFECT OF LONG-TERM ADMINISTRATION OF LOSARTAN, ANGIOTENSIN – 1 RECEPTOR ANTAGONIST, ON GEOMETRY OF CONDUIT ARTERIES IN WISTAR RATS AND SHR. R. Koprdová, M. Cebová, F. Kristek *Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic*

We evaluated the effect of AT_1 receptor blockade - losartan on blood pressure (BP), heart and structure of conduit arteries in normotensive Wistar rats and spontaneously hypertensive rats (SHR). Four groups of animals at the age of 4 weeks were used: 1) control Wistar rats, 2) SHR, 3) treated Wistar rats (20 mg kg^{-1} day $^{-1}$ b. w. by gavage), 4) treated SHR (20 mg kg^{-1} day $^{-1}$ b. w. by gavage). BP was measured on the tail artery using plethysmographic method. After 5 weeks animals were sacrificed, perfused (120 mm Hg) with glutaraldehyde fixative and processed according to standard electron microscopic procedure. Wall thickness (WT) and inner diameter (ID) of carotid artery (AC) and coronary artery (RS) were measured in light microscopy. Cross sectional area (CSA) and WT/ID (WD) were calculated.

Parameters	Wistar	Wistar+Los	SHR	SHR+Los
BP (mm Hg)	109±1.7	101±2.3 *	149±2.1 *	135±1.0^{+*}
HW/BW	4.6±0.3	3.0±0.1 *	5.8±0.2 *	4.2±0.1 $^{++}$
AC				
WT (μm)	27.0±1.2	21.3±0.6 *	33.8±0.7*	31.9±0.7 *
ID (μm)	743±23.7	825±23.1 *	714±21.2	792±18.1$^+$
CSAx10^{3}μm^2	64.8±2.3	56.4±1.8 *	79.1±1.5*	82.2±2.2 *
WD x 10^{-2}	3.7±0.3	2.6±0.1 *	4.8±0.2 *	4.1±0.2 $^+$
RS				
WT (μm)	10.4±0.8	9.4±0.8	13.8±0.6*****	10.9±0.4 $^+$
ID (μm)	217±10.2	221±14.7	212±5.4	251±10.6^{+*}
CSAx10^{3}μm^2	7.3±0.5	6.9±0.8	9.8±0.4 *****	9.0±0.5 *****
WDx10^{-2}	5.0±0.6	4.4±0.4	6.6±0.4 *****	4.5±0.3 $^+$

* Vs. Wistar rats, $^+$ SHR+Los vs. SHR

Long-term administration of AT_1 receptor blockator losartan reduced BP in Wistar rats and SHR and evoked regression of cardiac hypertrophy and remodelling of both conduit arteries in SHR. The study was supported by VEGA grant 2/3145/23, Slovakia.

DIFFERENT EFFECT OF NO DONORS ON GEOMETRY AND REACTIVITY OF CONDUIT ARTERIES IN SHR AND NO-DEFICIENT RATS F. Kristek, R. Koprdová, M. Cebová, J. Török
Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia

The aim of the study was to analyse whether increased NO level due to NO donors administration – pentaerythrityl tetranitrate (PETN) and molsidomine (Mols) is able to reverse morphological alterations in conduit arteries in two types of hypertension – spontaneously hypertensive rats (SHR) and rats in which hypertension was induced by N^G-nitro-L-arginine methyl ester (L-NAME) administration (50 mg/kg/day in tap water). Ten-week old rats were used: Wistar rats, Wistar+L-NAME, Wistar+L-NAME+PETN, Wistar+L-NAME+Mols, SHR, SHR+PETN, SHR+Mols. PETN (2x50 mg/kg/day in tap water) and Mols (2x100 mg/kg/day in tap water) were administered by a gavage. Experiments lasted 6 weeks. Blood pressure (BP) was measured on the tail artery using plethysmographic metod. For morphological study the rats were perfused with glutaraldehyde fixative (120 mmHg) and thoracic aorta, carotid artery, coronary artery, and basilar artery were excised and processed for electron microscopy. Geometry of the arteries was measured in light microscopy. For functional study isolated rings of TA and CA were used. BP was increased in L-NAME and SHR groups. NO donors decreased BP in L-NAME groups (it was still higher than in controls), not in SHR groups. In all arteries wall thickness, cross sectional area and wall thickness/inner diameter which were increased in both hypertensive models were reversed by NO donors only in L-NAME groups, not in SHR groups. Decreased endothelium-dependent relaxations of aorta precontracted by noradrenaline or phenylephrine were found in L-NAME group. In aorta and carotid artery from SHR, the endothelium-dependent relaxations to acetylcholine were not attenuated. The relaxation of arteries from SHRs, as well as the residual relaxations of arteries from L-NAME treated rats, were abolished by addition L-NAME to incubation medium. In NO-deficient hypertensive rats, the inhibitory effect of L-NAME on acetylcholine-induced relaxation was prevented by PETN and Mols administration. In conclusion: NO donors reversed pathological changes in cardiovascular system in NO deficient rats but not, however, in SHR. The results suggest that pathological background in these two types of hypertension is very probably different and accompanying functional and structural changes in SHR, contrary to NO-deficient hypertension, are not primarily evoked by deficiency of NO. The study was supported by VEGA grants No. 2/3145/25 and 2/3166/25 Slovakia

PROTECTIVE EFFECTS OF PENTOXIFYLLINE ON VASCULAR ENDOTHELIUM UNDER STRESS CONDITIONS V. Kristová*, Z. Pirnik, M. Mlynárik, A. Kiss, D. Ježová, **Department of Pharmacology, Faculty of Medicine, Comenius University, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia*

Stress is generally considered to be a risk factor of several diseases e.g. cardiovascular diseases. Some observations in humans and animals indicate vascular endothelial dysfunction in response to stress. New approaches in pharmacotherapy of cardiovascular disorders involve several groups of drugs with protective effect on vascular endothelium which may prevent or reduce cardiovascular risk. Pentoxifylline (PTX) is classified as a hemorrheological agent with vasodilator properties and other favorable effects on vascular wall including endothelium-protective effects (1).
The aim of this study was to investigate: 1. Relation between single exposure to an intensive stressor and potential endothelial damage, 2. Effect of PTX pretreatment on the endothelium and neuroendocrine activation during stress, 3. Comparison of PTX effects on these parameters between male and female rats. The rats were treated with saline or PTX (20 mg/kg s.c.) once daily for 7 days and then exposed to single immobilization stress for 20 or 120 min. Exposure of male rats to immobilization stress was followed by a rise in counts of circulating endothelial cells which was reduced by pretreatment with PTX. Statistical analysis by two-way anova showed significant differences for both stress exposure ($F=11.5$, $p < 0.001$) and treatment ($F=7.5$, $p < 0.01$). Concentration of von Willebrand factor rose in response to stress exposure ($F=12.7$, $p < 0.001$). The highest levels were observed shortly after beginning of immobilization stress (20 min, $q=7.0$, $p < 0.01$). Pretreatment with PTX did not reduce significantly the stress-induced rise in von Willebrand factor concentrations. Quantification of endothelial cells in female rats revealed significant rise in endothelaemia 24 h after immobilization stress ($p < 0.01$). PTX administration markedly reduced number of endothelial cells in blood. Stress-induced increase in hormone levels was reduced by PTX pretreatment in male rats. Significant inhibition was observed in plasma ACTH and corticosterone concentration in the adrenals. Treatment with PTX did not affect significantly hormone levels in female rats. These results have shown that protective action of PTX on vascular endothelium might be of benefit in prevention of stress-induced endothelial damage.

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RED WINE POLYPHENOLIC COMPOUNDS PREVENTED DEPLETION OF BRAIN MITOCHONDRIAL COENZYME Q IN SPONTANEOUSLY HYPERTENSIVE RATS. POSSIBLE MECHANISM OF BRAIN PROTECTION IN HYPERTENSION? J. Kucharská[1], Z. Sumbalová[1], A. Gvozdjáková[1], V. Bada[2], R. Andriantsitohaina[3], I. Bernátová[4], O. Pecháňová[4]. [1]*Pharmacobiochemical Laboratory and* [2]*3rd Dept. of Int. Med., Comenius Univ. Sch. Med. and* [4]*Inst. of Normal and Pathol. Physiology, SAS, Bratislava, Slovakia and* [3]*Université Louis Pasteur de Strasbourg, Illkirch, France,*

An imbalance between production of nitric oxide (NO), reactive oxygen species (ROS) and the level of protective antioxidants is involved in pathogenesis of hypertension. Resultant oxidative stress is supposed to play a role in higher incidence of cerebrovascular stroke and neurodegenerative diseases in hypertensive patients, antioxidants can be protective. Coenzyme Q (CoQ) besides its antioxidant function, which exerts in cooperation with α-tocopherol (α-toc), is a component of mitochondrial respiratory chain inevitable for ATP synthesis. CoQ_{10} deficiency was found in patients with hypertension. Consumption of red wine is associated with lower incidence of cardiovascular diseases. Aim of this study was: a/ to investigate endogenous concentrations of CoQ_9, CoQ_{10} and α-toc, activities of NO synthase (NOS) and to monitor blood pressure in a model of genetic hypertension in the rat, the spontaneously hypertensive rat (SHR), and b/ to study the effect of red wine polyphenolic compound (Provinol™, France). SHR and Wistar rats were treated with Provinol in dose 20 mg/kg/day in drinking water during 4 weeks. Systolic blood pressure was monitored by noninvasive tail pletysmography, antioxidants concentrations were determined in plasma, brain tissue and mitochondria by HPLC method, NOS activities were measured in the aorta and brain. SHR had significantly increased blood pressure and NOS activity in the aorta, decreased concentrations of CoQ_9 and α-toc in plasma, CoQ_9 and CoQ_{10} in brain mitochondria and α-toc in brain tissue. No significant changes were found in CoQ concentrations and NOS activities in brain tissue. Provinol treatment in SHR prevented increased blood pressure, restored decreased concentration of both CoQ homologues in brain mitochondria and increased NOS activity in aorta. It failed however to restore antioxidants in plasma and α-toc in brain tissue. Decreased concentrations of coenzyme Q in brain mitochondria in hypertension can be associated with oxidative stress and disturbances in brain bioenergetics, and may represent an early brain dysfunction. Consumption of red wine compounds with antioxidant and free radicals scavenging properties may be valuable in patients with hypertension to delay cognitive decline and dementia.
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THE ROLE OF REACTIVE OXYGEN SPECIES IN EXPERIMENTAL HYPERTENSION J. Kuneš[1,2], O. Pecháňová[2,3], Z. Dobešová[1,2], H. Rauchová[1,2], M. Vokurková[1,2], J. Zicha[1,2], [1]*CECR and* [2]*Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic,* [3]*Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic*

Hypertension is associated with an increased production of reactive oxygen species (ROS) and frequently also with an impaired bioavailability of the major vasodilator system – nitric oxide (NO). The dysbalance of vasoconstrictor and vasodilator systems is partly responsible for increased peripheral resistance in both human and experimental hypertension. The aim of our study was to evaluate the role of ROS and their interaction with NO in three different models of experimental hypertension (young and adult salt hypertensive Dahl rats, NO-deficient hypertensive rats chronically treated with L-NAME, young and adult SHR rats). The influence of ROS on physiological parameters was evaluated by chronic treatment with tempol or N-acetylcysteine. In some experiments the production of ROS was measured directly in aorta. Our data indicated that the exaggerated hypertensive response of immature Dahl rats to high salt intake is associated with a major impairment of NO-dependent vasodilation which fails to compensate adequately the enhanced sympathetic vasoconstriction. ROS production was lower and the impairment of NO-dependent vasodilation was absent in adult Dahl rats with a less severe form of salt hypertension. NO-deficient hypertension was accompanied by increased production of ROS as evidenced by increased lucigenin chemiluminescence in the aorta. The development of this form of hypertension was completely blocked by N-acetylcysteine treatment. Moreover, N-acetylcysteine partially decreased blood pressure in young SHR rats. In conclusion, ROS are important for both the induction and the maintenance of salt hypertension of Dahl rats, for the induction of NO-deficient hypertension as well as partially even for the development of genetic hypertension in SHR rats.
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ANGIOTENSIN II, OXIDATIVE STRESS AND INFLAMMATION IN HYPERTENSION V. Lahera, *Department of Physiology. Faculty of Medicine. University Complutense. Madrid. Spain.*

Hypertension is associated with functional and structural alterations of the arterial wall, which seem to be responsible for most of the vascular complications of this disease. Endothelial dysfunction has been proposed as the most important vascular alteration in hypertension, which leads to the development of arteriosclerosis. Hypertensive endothelial dysfunction is characterized by reduced endothelium-dependent relaxations and enhanced endothelium-dependent contractions. Reduced availability of nitric oxide (NO) is the major cause for reduced endothelium-dependent relaxations. Reduced expression of endothelial NO synthase (eNOS) and enhanced inactivation of NO by superoxide anions seems to major mechanisms contributing to reduced NO availability. In fact, increased expression of vascular NAD(P)H oxidase has been found in spontaneously hypertensive rats (SHR). In addition, increased production of cytokines and other mediators of inflammation has also been shown in hypertension. We recently found elevated plasma levels and vascular mRNA expression of IL1b and IL-6 in SHR, which were associated with enhanced expression of the transcription factor NFkB and reduced expression of its inhibitor IkB. Numerous studies in both hypertensive animals and patients showed that antihypertensive treatment was able to enhance endothelium-dependent relaxations and reduced arterial wall thickness. However, the reduction of hemodynamic stress does not seem to be the only mechanism responsible for the beneficial effects of antihypertensive drugs. Angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists (ARA II) have been demonstrated as the most efficacious antihypertensive drugs in ameliorating endothelial dysfunction and vascular remodeling. In a recent study we observed that chronic treatment with ARAII was able to enhance vascular eNOS expression. This effect was accompanied by reduction of vascular NAD(P)H oxidase expression and elevation of hepatic GSH/GSSG ratio, indicating that angiotensin II stimulates oxidative stress at both vascular an systemic level. In addition, treatment with ARAII reduced elevated plasma levels of cytokines as well as aortic mRNA expression of IL-1b, IL-6 and TNF-a. Furthermore, blockade of AT1 receptors also reduced NFkB and enhanced IkB. In conclusion, the participation of angiotensin II in oxidative stress and inflammatory process associated with hypertension appears to be crucial for the development of vascular complications of hypertension.

SEARCH FOR THE CAUSES OF VEIN GRAFT FAILURE IN CORONARY BYPASS SURGERY A. Loesch *Department of Anatomy, Royal Free and University College Medical School, London, UK*

This research focused on the structural and immunocytochemical features of human saphenous vein (SV) harvested for coronary artery bypass graft surgery (CABG). The differences between the conventionally harvested SV grafts, which show a relatively high failure rate, and grafts harvested with the non-invasive new 'no-touch' technique resulting in improved graft patency (1, 2) were disclosed. Drastic structural changes were observed in the conventionally harvested SV, which concerned endothelium, vascular smooth muscle cells (VSMCs), perivascular nerves and vasa vasorum (3, 4). These preparations displayed a decrease in eNOS (endothelial nitric oxide synthase - the enzyme involved in the synthesis of NO). VSMCs damaged by harvesting procedure displayed iNOS (inducible NOS). Electron microscopic examination suggested both proliferation and apoptosis of VSMCs in conventionally harvested SV (3). Light microscopy of conventional SV preparations revealed a presence of proliferating cell nuclear antigen-positive cells in the media (5), whilst confocal microscopy demonstrated abundance of 'dead' cells (calcein/ethidium stain); the latter might have also reflected an extensive increase in gap junctions. In conclusion, the conventional harvesting of SV for CABG rapidly stimulates iNOS in VSMCs damaged by harvesting. Thus the harvesting method influences iNOS expression in the SV at the time of grafting, which may be important for the graft patency. The possibility of apoptosis of VSMCs at the time of SV grafting is open for debate.

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CAPILLARY HISTOCHEMICAL AND SUBCELLULAR ALTERATIONS IN THE HEART OF NO-DEFICIENT RATS L. Okruhlicová, K. Dlugošová, M. Fialová, N. Tribulová, I. Bernátová[1], O. Pecháňová[1] *Institute for Heart Research, [1]Institute of Normal and Pathologic Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic*

Recent data provide increased evidence that morphologic alterations in the smallest segments of vascular system arterioles and capillaries represent the earliest form of heart damage in hypertension. The alterations are manifested by increase in arteriole wall-to-lumen ratio, insufficient capillary growth and rarefaction of capillaries, followed by endothelial dysfunction. Our previous study showed that chronic disturbances in NO production resulted in histochemical and subcellular alterations of rat myocardial tissue (1). The present study examined subcellular alterations of cardiac capillary endothelial cells in NO-deficient rats. To induce NO-deficient hypertension, male Wistar rats were treated with inhibitor of NO synthase L-nitroarginine methylester (L- NAME) 40 mg/kg for 4 weeks. Left ventricular tissue was processed for enzyme catalytic histochemistry as well as for ultrastructural examination of capillaries. Endothelial NO synthase/NADPH-diaphorase (NOS), and alkaline phosphatase (AP) together with dipeptidyl peptidase IV (DPP IV) as markers of capillary network were studied. Results showed reduced and/or abolished capillary NOS activity indicating impairment of NO production and capillary functional alterations. Likewise, micro-areas with lower and/or absent AP activity suggesting functional alterations of capillaries and their reduced myocardial density. In parallel, subcellular alterations of capillaries manifested by thickened basal membrane, protrusions and bridging of endothelial cells into the lumen, oedematous endothelial cells containing electrolucent nucleus, injured mitochondria and weakened endothelial junctions demonstrated impaired structural integrity and permeability of endothelial layer. On the other hand, structural markers of angiogenesis - pericyte-fibroblast interactions, endothelial cell migration, and capillaries profiles with a small lumen were observed. They indicated adaptation processes in the myocardium and improving of oxygen and nutrient transport to cardiomyocytes. Results point out that hypertension-related cardiomyopathy is accompanied with subcellular abnormalities of capillary endothelial cells indicating angiopathy as well as angiogenesis.

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THE MECHANISM OF ANTIHYPERTENSIVE ACTION OF MELATONIN IN SPONTANEOUSLY HYPERTENSIVE RATS: COMPARISON WITH SPIRONOLACTONE

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The aim was to investigate whether melatonin or spironolactone are able to reduce hypertension in spontaneously hypertensive rats (SHR), by means of the modulation of NO-synthase (NOS) activity, NOS expression and reactive oxygen species concentration indicated by nuclear factor-κB (NF-κB) expression. Three groups of adult SHR were investigated: Control group (placebo, n=7), melatonin (10 mg/kg/24h melatonin, n=8) and spironolactone (200 mg/kg/day spironolactone, n=6) group. During five weeks of treatment, systolic blood pressure (SBP) was measured by tail-cuff plethysmography. Samples were taken from the left ventricle (LV) and from the kidney. The NOS activity was investigated on the base of L-citrulline formation from radioactive L-arginine. The protein expressions were determined using Western-blotting. In the control SHR group, SBP increased progressively (177±3 mmHg). Although a reduction of blood pressure was observed in both melatonin (74%) and spironolactone (80% of the control) treated animals, only melatonin was able to enhance the NOS activity (121% and 119% in the kidney and LV, respectively) and to decrease the NF-κB expression (71%, 71%). An increase in NOS expression after melatonin treatment was observed only in the LV (129%). Spironolactone changed neither the NF-κB expression nor the NOS activity, although the NOS expression was enhanced in both tissues investigated (125%, 134%). In conclusion, we suppose that the decrease in the reactive oxygen species concentration is more important for enhancing the NO-synthase activity, than the increase in NO-synthase protein expression. The antioxidant properties of melatonin may thus contribute to its antihypertensive effect.

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ANTIOXIDANT EFFECT OF ANTIHYPERTENSIVE THERAPY IN EXPERIMENTAL HYPERTENSION

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Data from numerous studies underline the importance of imbalance between the production of antioxidants and reactive oxygen species (ROS) for the development and maintenance of cardiovascular diseases including hypertension. The aim of our study was to investigate the antioxidant effect of different antihypertensive drugs on the prevention of blood pressure increase in spontaneous and L-NAME-induced hypertension. Young 5-week-old SHR were treated with ACE inhibitors (captopril, enalapril), aldosterone receptor blocker (spironolactone), diuretics (hydrochlorothiazide, indapamide) or antioxidants (N-acetylcysteine, melatonin) during 4-6 weeks. In the model of L-NAME-induced hypertension, the drugs were given to adult 12-week-old Wistar rats simultaneously with the inhibitor of nitric oxide synthase L-NAME. Systolic blood pressure (SBP) was measured by tail-cuff plethysmography. Nitric oxide synthase (NOS) activity and concentration of conjugated dienes (CD) were determined in the left ventricle and kidney in which protein expression of eNOS and NF-κB were measured. All substances used in the study partially attenuated SBP rise in young SHR and in L-NAME treated rats. Captopril (100 mg/kg/day) and indapamide (1 mg/kg/day) had more pronounced effect on NOS activity increase and CD reduction than enalapril and hydrochlorothiazide, respectively. Antioxidants, N-acetylcysteine (1.5g/kg/day) and melatonin (10 mg/kg/day) increased NOS activity and decreased CD concentration in both tissues investigated, but their effects were more pronounced in SHR than in L-NAME treated animals. In conclusion, besides the direct effect on Ang II production and sodium reabsorpcion, the thiol group of captopril and the indoline group of indapamide may be responsible for antioxidant effect of these drugs. N-acetylcysteine and melatonin could interfere better with ROS generation in SHR than in L-NAME treated rats.

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DUAL ROLE OF NO AND OXYGEN RADICALS IN ISCHEMIA/ REPERFUSION INJURY IN THE ADAPTED AND NON-ADAPTED HEART T. Ravingerová, E. Andelová, M. Pintérová, R. Važan[1], O. Szárszoi[2], F. Kolář[2] *Institute for Heart Research, Slovak Academy of Sciences, [1]Department of Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic; [2]Institute of Physiology, Academy of Sciences of the Czech Republic and Centre for Cardiovascular Research, Prague, Czech Republic*

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 (non-stressed) heart and in the myocardium adapted to short-term and long-lasting stressful stimuli. In a setting of test IR in the Langendorff-perfused rat hearts, acute blockade of NO production by NO synthase (NOS) inhibitor L-NAME suppressed severe ventricular arrhythmias induced by either regional (LAD coronary artery occlusion) or global IR in non-adapted hearts (arrhythmia score 0.9 ± 0.2 and 2.7 ± 0.3 vs 4.0 ± 0.3 and 3.8 ± 0.2 in the respective 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 ± 5.1 % in the controls to 28.4 ± 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 hearts subjected to ischemic preconditioning (IP) by two brief episodes of IR, prior to test IR. Chronic intermittent high altitude (IHA) hypoxia, despite its negative cardiopulmonary effects, leads to long-term adaptation that renders rat hearts more resistant to all manifestations of IR, similarly to IP (1). However, different from IP, L-NAME treatment did not affect reduced arrhythmogenesis in the IHA hearts. Increased production of oxygen free radicals (OFR) was observed during ischemia, IP and IHA hypoxia. Whereas reperfusion arrhythmias were effectively attenuated by antioxidant treatment (melatonin, tempol or N-acetylcysteine) given before ischemia in the non-adapted hearts, antiradical interventions failed to protect the adapted hearts against IR insult and blunted antiarrhythmic protection induced by IP and IHA hypoxia. It is concluded that NO and OFR play a dual role in the heart. Being deleterious in the intact myocardium exposed to prolonged ischemia and contributing to lower ischemic tolerance in the non-adapted heart, they might be also implicated in cardioprotection conferred by both, short-term and long-term adaptation as important signaling molecules.

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NATURAL ANTIOXIDANTS AND THEIR CYTOPROTECTIVE EFFECTS

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Antioxidants are a group of substances which, when present at low concentrations, in relation to oxidizable substrates, significantly inhibit or delay oxidative processes, while often being oxidized themselves. In recent years there has been an increased interest in the application of natural antioxidant to medical treatment as information is constantly gathered linking the development of human diseases to oxidative stress. The generally accepted hypothesis is that in any biological system a balance must be maintained between the formation of reactive oxygen species (ROS) and their removal because their increase produces toxic effects on cellular metabolism. The beneficial effects of fruits, vegetables or red wine reported, may be in part explained by their content in polyphenols with a multitude of biological activities, including antioxidant and free radical-scavenging properties, anti-aggregatory platelet property and inhibition of vascular smooth muscle cell proliferation. Moreover, it has been demonstrated that plant-derived polyphenols prevent the development of hypertension and myocardial fibrosis. Recently, we tested the hypothesis that administration of Provinol™ (PV), a red wine polyphenol, prevents the development of nephrotoxicity induced by immunosuppressive treatment with Cyclosporine A (CsA) in experimental conditions. We used 3 groups of rats: control; group treated with CsA (15mg/Kg/day); group treated with CsA plus PV (40mg/Kg/day). CsA treatment produced a significant increase in systolic blood pressure, did not affect urinary output but caused a significant decrease in creatinine clearance. Moreover, CsA induced histological alterations including tubular injury and interstitial fibrosis. These effects were associated with an increase in conjugated dienes, inducible NO-synthase (iNOS) and nuclear transcription factor NF-kB. PV alone did not affect these parameters but combined with CsA prevented both functional and structural alterations induced by immunosuppressive treatment. The protective mechanism of PV involved reduction of both oxidative stress and increased iNOS expression via the NF-kB pathway. These results show that PV administration is protective against CsA-induced nephrotoxicity and provide a pharmacological basis for the beneficial effects of natural antioxidants against renal damage associated with immunosuppressive agents.

EFFECT OF CHRONIC INDAPAMIDE, HYDROCHLOROTHIAZIDE AND CAPTOPRIL TREATMENT ON RELAXATION OF FEMORAL ARTERY IN SPONTANEOUSLY HYPERTENSIVE RATS

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Essential hypertension is associated with alterations in endothelial function. Various antihypertensive drugs are supposed to restore normal function leading to the improvement of vasorelaxation. The purpose of this study was to investigate the effect of chronic indapamide, hydrochlorothiazide, captopril and indapamide-captopril combination treatment on the endothelial function in SHR by evaluating the endothelium-dependent relaxation of the femoral artery and the blood pressure development.

Four groups of 6-week-old male SHR were orally treated with antihypertensive drugs: indapamide (1 mg/kg/day), hydrochlorothiazide (10 mg/kg/day), captopril (10 mg/kg/day) and indapamide-captopril combination for six weeks and compared with controls. The endothelium-dependent relaxations were tested using the Mulvany myograph. Systolic blood pressure was measured by tail-cuff plethysmography in conscious rats.

Indapamide (I), hydrochlorothiazide (HCT), captopril (C) and indapamide-captopril combination (I+C) treatment significantly decreased the blood pressure rise in young SHR (I: 157±4, HCT: 154±3, C: 137±2, I+C: 121±1 mmHg) in comparison with untreated animals (171±1 mmHg). Indapamide treatment significantly increased acetylcholine-induced relaxation responses of the femoral artery by 9% compared to the control group and by 14% compared to the HCT group. Captopril treatment failed to affect the relaxation of the femoral artery and simultaneous treatment with indapamide-captopril combination increased relaxation responses similarly as indapamide alone (by 9%).

While indapamide and captopril had the additive effect on the prevention of the blood pressure increase, relaxation responses of the femoral artery were improved only by indapamide treatment. It is supposed that indapamide is able to elevate NO synthase activity leading to increased endothelium-dependent relaxation responses.

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THE EFFECTS OF QUERCETIN, CHRYSIN AND MORIN ON THE DEVELOPMENT OF SPONTANEOUS HYPERTENSION AND NO GENERATION IN THE HEART AND KIDNEY. Z. Sulová Jr.[1,2,3], A Breier[2], S. Kojšová[1], L. Jendeková[1], P. Janega[1,4], Z. Sulová[2], E. Šturdík[3], O. Pecháňová[1], *[1]Institute of Normal and Pathological Physiology, [2]Institute of Molecular Physiology and Genetic, Slovak Academy of Sciences, [3]Faculty of Food and Chemical Technology, Slovak Technical University, [4]Medical School, Comenius University, Bratislava, Slovak Republic*

Epidemiological studies have suggested that diet rich in natural polyphenols can reduce the risk of cardiovascular diseases. Mechanisms that have been proposed to explain the prevention of cardiovascular diseases by polyphenols, including increased of NO generation and decreased of reactive oxygen species, are extensively studied. The aim of our work was to determine the effects of flavonoids: quercetin, morin and chrysin on the development of blood pressure in spontaneously hypertensive rats (SHR) and to evaluate their effects on NO synthase (NOS) activity and concentration of reactive oxygen species. Young 4-week-old male SHR were treated with quercetin, morin or chrysin in the dose 20 mg/kg/day for four weeks. Systolic blood pressure (SBP) was measured by tail-cuff plethysmography. Total nitric oxide synthase (NOS) activity was determined by measuring the formation of L-[^{3}H] citrulline from L-[^{3}H] arginine in the left ventricle and kidney. Conjugated diene (CD) concentrations were detected spectrophotometrically in the same tissues. Chronic quercetin, morin and chrysin treatment partially attenuated SBP rise in young SHR (140±3, 139±3 and 146±3 mmHg, respectively, vs. 157±4 mmHg in untreated animals). All flavonoids investigated significantly increased NOS activity in the kidney, while they failed to modify NOS activity in the left ventricle. Accordingly, flavonoids decreased reactive oxygen species (measured as CD concentration) in the kidney and did not modify the CD concentration in the left ventricle.
In conclusion, both increased NOS activity and ROS reduction may be responsible for preventive effect of flavonoids on the blood pressure increase in SHR.
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EFFECT OF MELATONIN ON ENDOTHELIAL FUNCTION OF CONDUIT ARTERY IN RAT WITH EXPERIMENTAL HYPERTENSION J. Török, S. Líšková, Ľ. Paulis[1], K. Krajčirovičová[1], O. Pecháňová, F. Šimko[1] *Institute of Normal and Pathological Physiology, Slovak Academy of Sciences and [1]Department of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia*

The aim of this study was to determine the effect of a long-term administration of melatonin, a hormone synthetized in the pineal gland, on systolic blood pressure (SBP) and the reactivity of isolated thoracic aorta from nitric oxide (NO)-deficient hypertensive and spontaneously hypertensive rats (SHR). Five groups of rats were used: 1)control (normotensive) rats; 2) rats treated with nitro-L-arginine methylester (L-NAME, 50mg/kg/day) - NO-deficient hypertensive rats; 3) rats treated simultaneously with L-NAME (50m/kg/day) and melatonin (12mg/kg/day); 4) SHR; 5) SHR treated with melatonin (12mg/kg/day). The substances were given to tap water for 5 weeks. SBP was measured on the tail artery using plethysmographic method. Isolated rings of thoracic aorta were suspended in organ chamber and connected to a force transducer for the recording of isometric tension. Melatonin produced significant reduction of SBP in both SHR and NO-deficient hypertensive rats. Increased SBP was accompanied by an increase of the left ventricle/body weight ratio in both NO deficient and SHR compared with age-matched normotensive controls indicating cardiac hypetrophy. Melatonin decreased this ratio in NO-deficient hypertensive animals. Endothelium-dependent relaxation of aortic rings from NO-deficient hypertensive rats was markedly and from SHR only slightly reduced. The inhibitory effect of L-NAME on acetylcholine-induced relaxation was partially reversed by simultaneous treatment of rats with melatonin. Also in aortic rings from SHR the maximal relaxation to acetylcholine was improved to the control rats level. Noradrenaline produced concentration-dependent contraction of the aorta that was comparable among control normotensive rats, SHR and L-NAME-treated rats. Melatonin treatment in SHR and L-NAME-treated rats was without significant effect on noradrenaline contraction. The results showed that melatonin exerted an antihypertensive action in both models of experimental hypertension. Beneficial effect of melatonin on the acetylcholine-induced relaxation in the aorta, at least in NO-deficient rats, could be caused by activating of vascular NO pathway.
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MYOCARDIAL GAP JUNCTION REMODELLING IN NO-DEFICIENT HYPERTENSION N. Tribulova, L. Okruhlicova, M. Fialova, P. Weismann[1], I. Bernatova[2], O. Pechanova[2] *Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovakia, [1]Institute of Anatomy, Medical Faculty, Bratislava, Slovakia, [2]Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia.*

Previously we have shown that chronic disturbances in NO production result in myocardial remodelling and marked subcellular changes of cardiomyocytes as well as capillaries (1,2). Cardiac gap junctions play an important role in intercellular electrical coupling and myocardial synchronisation. Since hypertension is known to increase a risk for severe cardiac arrhythmias, aim of the study was to examine whether myocardial gap junctions and susceptibility to arrhythmias are altered in heart of rats suffering from NO-deficient hypertension. Wistar rats were administered with L-NAME (40mg/kg) for a period of 4 weeks. Thereafter, isolated heart was perfused in Langendorff mode with oxygenated Krebs-Henseleit solution followed by K^+ deficient once, to induce VF. Heart tissue sampling was performed for ultrastructure examination and "in situ" immunodetection of major gap junction protein, connexin-43, using primary monoclonal mouse anti-Cx43 antibody and secondary goat antimouse IgG antibody conjugated to FITC. Intensity of fluorescence signal was evaluated using image analySIS program and expressed as a proportion of the total tissue area. Ultrastructure and immunolabeling showed that NO-deficient hypertension is accompanied by gap junction remodelling, i.e. abnormal distribution of gap junctions showing enhancement of lateral connections. Moreover, connexin-43 expression was locally decreased corresponding to the area of fibrosis and/or cardiomyocyte injury. Patchy loss of connexin-43 immunoreactivity was more pronounced during perfusion of the heart with K^+ deficient solution prior occurrence of VF. Compared to controls, there was a sooner onset of transient arrhythmias and significantly higher incidence of sustained VF in NO-deficient rats. In conclusion, NO-deficient hypertension is associated with gap junction remodelling and heterogeneously decreased myocardial cell to cell coupling, which deteriorate due to acute pathophysiological conditions. These changes may result in conduction slowing and facilitate development of malignant arrhythmias.
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REGULATION OF CARDIAC NA,K-ATPASE BY NITRIC OXIDE IN VARIOUS MODELS OF HYPERTENSION N. Vrbjar, J. Vlkovičová, V. Javorková, O. Pecháňová[1] *Institute for Heart Research, Department of Biochemistry, Slovak Academy of Sciences, Bratislava, Slovak Republic; [1]Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic*

The Na,K-ATPase is hypothesized to be involved in systemic vascular hypertension through its effects on smooth muscle reactivity and cardiac contractility. The aim of the present study was the investigation of regulatory role of nitric oxide (NO) on functional properties of the cardiac Na,K-ATPase during hypertension. Three various animal models of hypertension were studied. The first was represented by spontaneously hypertensive male rats (SHR) with increased synthesis of NO by 60% (A). The second group of SHR revealed a decreased synthesis of NO by 40% (B). In the third model (C), the hypertension was induced by application of an inhibitor of NO synthesis, L-NAME, in the dose of 40mg/kg/day resulting in depression of NO synthesis by 70%. Studying the utilization of energy substrate ATP we observed higher Na,K-ATPase activity in the whole concentration range of ATP for the group A. Evaluation of kinetic parameters in this group revealed the increased value of V_{max} (by 37%) and increased affinity of the ATP binding site as indicated by the lowered K_m value (by 38%). In the group B we found an inhibition of the enzyme at all ATP concentrations resulting in decreased V_{max} (by 30%) with no significant change of K_m value as compared to respective controls. In the group C, the Na,K-ATPase activity was not affected throughout the investigated range of ATP resulting in no change of V_{max} as well as the K_m as compared to respective controls. During the activation with Na^+ we observed a stimulation mainly in higher concentrations of cofactor yielding increased V_{max} by 64% and increased K_{Na} by 106% in the group A. In group B we found decreased activity in the whole concentration range of NaCl resulting in decreased V_{max} by 40% and increased K_{Na} by 38% . In the group C the enzyme activity was significantly depressed at lower concentrations of NaCl, showing unchanged V_{max} with increased K_{Na} by 50%. The above data indicate a positive role of increased NO-synthesis in improved utilization of ATP as well as enhanced binding of Na^+ by the cardiac Na,K-ATPase. On the other hand the decreased synthesis of NO is followed by deteriorated affinity of the enzyme to Na^+ in hypertensive rats.
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VASOACTIVE BALANCE IN EXPERIMENTAL HYPERTENSION: THE ROLE OF ENHANCED Ca^{2+} INFLUX

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The dysbalance of vasoconstrictor and vasodilator systems is partly responsible for increased peripheral resistance in both human and experimental hypertension. Our studies carried out in three different forms of experimental hypertension (salt hypertension of Dahl rats, NO-deficient hypertension induced by chronic L-NAME treatment, spontaneous hypertension of SHR) indicated augmented sympathetic component of blood pressure (BP) in all three forms of experimental hypertension. The magnitude of this component is also decisive for BP changes elicited by chronic captopril treatment in SHR or by antioxidant N-acetylcysteine administration in Dahl rats. Furthermore, NO-dependent vasodilation fails to compensate enhanced sympathetic vasoconstriction not only in L-NAME hypertension but also in SHR and Dahl rats. In all three forms of experimental hypertension sympathetic hyperactivity and/or relative NO deficiency is accompanied by enhanced blood pressure response to acute nifedipine administration. Nifedipine-induced BP change is negligible in normotensive animals but rises proportionally to basal BP elevation. Although several mechanisms were proposed to explain the augmented Ca^{2+} entry through voltage-dependent Ca^{2+} channels in hypertensive animals, enhanced proportion of tonic sympathetic vasoconstriction seems to be the most probable reason. This is further supported by our experiments on isolated blood vessels in which high noradrenaline concentrations open voltage-dependent Ca^{2+} channels and nifedipine lowers wall tension proportionally to initial tension. In conclusion, the augmented sympathetic vasoconstriction, which is characteristic for most forms of experimental hypertension, is mediated by enhanced Ca^{2+} entry through dihydropyridine-sensitive Ca^{2+} channels. This is further aggravated by the relative insufficiency of NO to open Ca^{2+}-dependent K^+ channels in order to secure sufficient vasodilation.

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REACTIVE OXYGEN SPECIES AND NO: FRIENDS OR FOE IN ADAPTATION OF THE MYOCARDIUM TO ACUTE DIABETES

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Background: Generation of reactive oxygen species (ROS) in respiratory chain of acute diabetic (DIA) heart induces perturbances in mitochondrial (MIT) state 3 & 4 QO_2, RCI, phosphorylation rate and CoQ_{10} oxidation ($p<0.05$-0.001), but affects little the ADP:O ratio. However, the slowed down oxid./phosp. and EOT-transport are compensated by increased MIT Mg-ATPase activity and by augmented energy delivery to cytoplasm, via elevated transmembrane permeability pores formation (all $p<0.05$) (1). Objective of the present study is the elucidation of real roles that play ROS and NO-synthase (NOS) in the MIT of DIA hearts. Experimental: 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 acceptor respectively. Total MIT NOS activity was estimated by conversion of [^{3}H]-arginine to [^{3}H]-citrulline. Results and Discussion: DIA heart MIT exhibited increased MF ($p<0.01$), decreased MP ($p<0.001$) coupled with enhanced capability to maintain it, no considerable increase in conjugated dienes formation in MIT membrane lipids and lower total NOS activity ($p<0.006$). Linear regression analysis revealed significant association ($p<0.05$, $r=0.67$) between MF increase and MP decrease in DIA MIT. Conclusions: ROS–induced attack to heart MIT in acute DIA proved to be restricted to significant, but not critical slow down of electron-oxygen transport and oxid./phosph. A probable link between ROS action and NOS depression may be not excluded, but still it has to be verified. Other changes observed may represent parts of endogenous protection and adaptation mechanisms.

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ENDOTHELIAL DYSFUNCTION IN EXPERIMENTAL MODELS OF DIABETES AND ISCHEMIA/REPERFUSION-EFFECT OF ANTIOXIDANTS

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Endothelial dysfunction occurs in various diseases such as hypertension, atherosclerosis, diabetes and also in conditions like of ischemia/reperfusion. Increased amount of reactive oxygen species (ROS) has been accpeted as the key event in the pathogenesis of endothelial dysfunction. Impaired endothelium-dependent vasodilatation is often the first sign of adverse cardiovascular events and can predict their long-term outcome.

In this work we present our results from several studies investigating the effect of streptozotocin (STZ)- induced diabetes or ischemia/reperfusion (I/R) on the functional state of the endothelium in male Wistar rats. Diabetes was induced by i.v. administration of STZ in the dose of 55 mg/kg and lasted from one to eight months. Reversible impairment of endothelial function, manifested by decreased relaxation response of preparations of the aorta and superior mesenteric artery (SMA) to acetylcholine, was observed already one month after STZ administration. I/R, induced by oclusion of SMA and abdominal aorta evoked changes in endothelial relaxation of arterial preparations (the aorta, SMA including the first-order arteries) to acetylcholin. Endothelial dysfunction was accompanied with microstructural changes of abdominal aorta and increased production of thiobarbituric acid reactive substances (lipid peroxidation marker). Moreover, increased production of ROS in reperfused arterial tissue was detected also by luminol (400μmol/l) enhanced chemiluminiscence. In the early phase of reperfusion, confocal microscopy with dihydroethidium showed increased production of superoxide anion ($O_2^{\cdot-}$) in SMA. Antioxidants used in study (stobadine, 2,3-dihydromelatonin) were able to prevent, at least to some extent, changes caused by diabetes and I/R. All these data support the tenet that the endothelial dysfunction of SMA and the aorta of STZ and I/R rats may be mediated by effects of ROS which oppose the vasorelaxant mechanism. STZ-diabetes and I/R can be consider suitable experimental models to investigate pharmacological effects of antioxidants.

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