

decrease of endoglin expression, which was shown in atherosclerosis in mice. In our prospective study, statin treatment will be evaluated, in order to elucidate whether endoglin expression as possible atherosclerosis protective marker can be increased/restored as demonstrated in experimental atherogenesis in vivo. The study was supported by grant from The Grant Agency of Charles University in Prague number 300811/C and grant SVV/2012/265003.

27. Expression of melatonin receptors in mesenteric arteries of hypertensive rats

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Hypotensive effects of melatonin (MEL) were demonstrated in spontaneous hypertensive rats (SHR). Mesenteric arteries (MA) contribute to hypertension and are remodeled in hypertensive individuals. MEL may exert its hypotensive effects via membrane receptors, MT₁ and MT₂, but their localization and function in the vessel wall remains mostly unknown. This study aimed to evaluate expression and localization of MT₁ and MT₂ in the rat MA in comparison to thoracic aorta (TA). Male Wistar (WKY) and SHR rats were treated for 4 weeks with MEL (40 µg/ml) in drinking water. MT₁ and MT₂ mRNA expression in MA and TA was studied by real-time quantitative PCR and localization in the vessel wall was analyzed by immunofluorescence microscopy on paraffin-embedded tissue sections. Higher expression of MT₁ receptor mRNA was demonstrated in MA in comparison to TA in WKY (p<0.001) and SHR (p<0.001) rats, while MT₂ mRNA was absent from all samples. In all vessels of WKY and SHR rats, MT₁ protein was localized to the tunica adventitia and the perivascular tissue. In the MA, MT₁ was additionally found in the tunica media, corresponding to the increased MT₁ mRNA expression. A lower (p<0.001) MT₁ mRNA expression in MA was found in SHR (15.3±1.9ΔCT) in comparison to WKY rats (12.5±0.8ΔCT). MEL treatment further decreased (p<0.05) MT₁ mRNA level in SHR MA. In conclusion, expression level and localization of MT₁ receptors vary among different type of vessels, and in MA expression levels are significantly altered by hypertension and upon MEL treatment. Localization data suggest that MEL contributes to contraction/relaxation in the tunica media, and protection and remodeling in the tunica adventitia and the perivascular tissue. Supported by APVV-0150-10, NOREG and FFG/Bridge 818 094 grants.

28. Endothelial response as a possible cause of difference in atherosclerosis susceptibility in mice

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Generally, mice do not develop atherosclerotic lesions on a low-fat diet (atherosclerosis resistance), however, it was demonstrated that C57BL/6J mouse strain is the most

susceptible, while C3H/HeJ is the most resistant. Endoglin, an important member of TGF-β signaling pathway, endothelial nitric oxide synthase (eNOS), with its ability to produce nitric oxide and reduce immune-cell activation, and heme oxygenase (HO-1), an important enzyme of heme catabolism that exerts anti-atherogenic effects, represent potential endothelial atheroprotective markers. In contrast, endothelial-specific molecules - vascular cell adhesion molecule (VCAM-1) and intercellular cell adhesion molecule (ICAM-1) strongly participate in leukocyte adhesion to endothelium and contribute to lesion formation. In this study, we hypothesized, whether cholesterol-rich diet affects aortic endothelial expression of atheroprotective and proatherogenic markers, that might be responsible for distinct predisposition to atherosclerosis in these mice. Female C57BL/6J mice (n=16) and C3H/HeJ mice (n=16), both divided into two groups, sustained on either chow or cholesterol (1%) diet for 8 weeks after the weaning. Biochemical analysis of blood cholesterol fractions and Western blot analysis of endoglin, eNOS, HO-1, VCAM-1, ICAM-1 expressions in aorta were performed. Atherogenic index (total cholesterol / HDL cholesterol ratio) was significantly raised in both mouse strains after cholesterol diet, with stronger impact in C3H/HeJ mice. Despite the higher atherogenic index in C3H/HeJ mice, Western blot analysis showed significantly increased expressions of endothelial-protective endoglin, HO-1, eNOS and significantly reduced expression of pro-inflammatory VCAM-1, ICAM-1 in C3H/HeJ mice after cholesterol diet in comparison with C57BL/6J mice. We propose that different reaction of endothelium to cholesterol-rich diet might represent a potential cause of difference in atherosclerosis susceptibility between C3H/HeJ and C57BL/6J mouse strains. The study was supported by grant from The Grant Agency of Charles University in Prague number 300911/C and grant SVV/2012/265003.

29. The role of Transient Receptor Potential Melastatin-4 (TRPM4) in vascular biology

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The transient receptor potential melastatin-4 (TRPM4) is a calcium-activated monovalent cation channel. TRPM4 has a major role in pressure-induced depolarization and vasoconstriction in intracranial arteries. Our aim was to study the regulatory function of TRPM4 in basilar (isometric measurements, precontraction by about 5 mN) and mesenteric (isobaric conditions, at 80 mmHg) arteries. Arteries were isolated from Wistar-Kyoto (WKY) rats and experiments were performed in oxygenated Krebs buffer. Initial diameter of the mesenteric arteries was 325±18 µm (n=9). Extracellular K⁺ (120 mM) evoked a vasoconstriction (decrease in diameter from 316±30 µm to 155±40 µm, n=4, p=0.03), similarly to norepinephrine (10 µM, decrease from 387±17 µm to 127±18 µm, n=5, p<0.01). Arteries were pre-constricted with norepinephrine and treated with the TRPM4 channel inhibitor 9-phenanthrol (1 µM-300 mM), which evoked a dilation (increase of diameter from 206±22 µm to 390±10 µm, n=5, p<0.01). Extracellular K⁺ mediated constriction was also inhibited by 9-phenanthrol. Extracellular K⁺ (60 mM)

evoked an increase in contractile force in the basilar arteries of the rat (contractile force from 2.6 ± 0.9 mN to 9.3 ± 1.3 mN, $n=4$, $p<0.01$). This extracellular K^+ mediated constriction was also inhibited by 9-phenanthrol (contractile force from 8.3 ± 1.4 mN to 2.9 ± 0.9 mN, $n=4$, $p=0.02$). Our data suggest that TRPM4 has a major role in the modulation of contraction followed by various agonist stimuli in different arteriolar beds of the rat. TRPM4 may participate in pathological conditions characterized by increased arteriolar tone, such as hypertension.

30. The effect of omega-3 fatty acids on expression of connexin40 in aorta of Wistar and hereditary hypertriglyceridemic rats during inflammation

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Gap junctions (GJ) are intercellular channels consisting of transmembrane proteins, connexins (Cx). GJ role is to provide direct passive communication between adjacent cells transporting low molecular weight compounds (metabolites and ions) and thus maintain cell homeostasis and synchronization of cell function in tissue. Cx expression may also be affected by pathological conditions. Hypertriglyceridemia (HTG) and inflammation are atherogenic risk factors. Therefore, we studied their effect on the expression of Cx40 in the endothelium and media of rat aorta. Due to the multiple protective effects of Ω -3 fatty acids (FA) on the cardiovascular system, including anti-inflammatory, we also studied whether FA can affect the expression of Cx40 (typical mainly for vascular endothelium) and thus the function of aorta in conditions of inflammation and HTG. We used adult Wistar rats (W) and hereditary hypertriglyceridemic ones (hHTG). In both models, we had groups injected with a single dose of lipopolysaccharide (LPS, 1mg/kg, ip). Endotoxemic rats were fed Ω 3-FA (30mg/kg/day/10 days). Spatial expression of Cx40 on cryostat sections of the aorta was detected using immunofluorescent (IF) confocal microscopy. IF signal of Cx was evaluated by morphometric image analysis and spatial Cx expression was presented by the number of IF plaques and their extent per unit area of endothelium and media. We analyzed NO-dependent relaxation of aorta and activities of NO synthase in aorta and NAGA (lysosomal enzyme, a marker of cell damage) in plasma. We also determined levels of C-reactive protein (CRP) and malondialdehyde (MDA) as markers of inflammation and oxidative stress in the plasma. Our results showed that HTG resulted in double increase in expression of Cx40 in both endothelium and media when compared to W. LPS significantly affected Cx40 expression mainly in endothelium of W and hHTG compared to LPS-free controls. Endothelial Cx40 expression was increased in W while decreased in hHTG. In contrast, LPS had no effect on Cx40 in media of aorta of W, but it reduced Cx40 in media of hHTG. Effect of Ω 3-FA on expression of Cx40 was more marked in W, where we observed a decrease in Cx expression. NO-dependent relaxation of aorta was impaired in hHTG rats compared with W. LPS deteriorated aortic relaxation in hHTG and treatment of inflammation with Ω 3-FA resulted in improved

function. Administration of Ω 3-FA did not affect relaxation of the aorta in W. Our results showed that both risk factors for atherogenesis, HTG and LPS affected the function of the aorta, increased levels of CRP and MDA as well as NAGA activity in plasma and NOS in the aorta. Ω -3 fatty acids positively influenced the function of the aorta and Cx40 expression. The results suggest that gap junctions consisting of Cx40 may be involved in the protective effect of fatty acids on the aortic function modulated by endothelium during inflammation. Supplied by VEGA 2/0108/10.

31. Whey proteins influence blood clotting parameters in hyperglycemic rats

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The aim of the study was to evaluate the effect of whey proteins concentration (WPC 80), at different doses, on blood clotting parameters in hyperglycemic rats. Blood samples were collected from 14 - month rats with hyperglycemia which had been taking whey proteins during 7 or 14 days. We examined basic parameters of coagulation profile, including: PLT (platelet count), PT (prothrombin time), APTT (activated partial thromboplastin time), TT (thrombin time) and fibrinogen level. We observed no effect on hemostatic parameters, excluding PLT, TT and level of fibrinogen. We found that the application of dietary proteins elongated the TT. The thrombin time prolongation was observed regardless of the dose of protein and time of exposition. The time required for plasma fibrinogen to form thrombin, measured as the time for clot formation after exogenous thrombin is added to citrated plasma. The difference in time indicates an abnormality in the conversion of fibrinogen (a soluble protein) to fibrin (insoluble protein). In addition we observed increased level of fibrinogen as compared to control group of animals. We point out for minor, insignificant, differences in the PLT level in rats, after high dose of proteins. This study showed that protein-rich diet may be prophylaxis against deep vein thrombosis in patients with hyperglycemia.

32. Effects of hydrogen-peroxide and MPO in the rat basilar artery

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Increased expression of myeloperoxidase (MPO) plays a role in the development of cardiovascular and cerebrovascular diseases. MPO is an important enzyme that catalyzes the formation of reactive molecules such as hypochlorous acid from hydrogen peroxide (H_2O_2). The aim of this study was to characterize the vasoactive effects of H_2O_2 and MPO in isolated arteries. Arteria basilaris was isolated from the rat. Arterial rings (3-5 mm in length) were fixed on an isometric contractile measurement system. H_2O_2 evoked changes in contractile force (in mN) of precontracted vascular rings were measured in the presence