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P10.04: INFLUENCE OF 1,25(OH)₂D₃ (VITAMIN D) ON RESISTANCE ARTERY CONTRACTILITY

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P10.01

INVOLVEMENT OF CALCIUM-CALMODULIN DEPENDENT PROTEIN KINASE II ON ENDOTHELIN RECEPTOR EXPRESSION IN CEREBRAL ARTERIES OF RAT

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Background. Experimental cerebral ischemia and organ culture of cerebral arteries result in enhanced expression of endothelin ET_B receptors in smooth muscle cells. The present study was designed to evaluate the involvement of calcium-calmodulin dependent protein kinase (CAMK) on the expression of endothelin receptors.

Methods. Rat basilar arteries were incubated for 24 hours with and without the CAMK inhibitor, KN93, or the ERK1/2 inhibitor, U0126. The contractile responses to endothelin-1 (ET_1 ; ET_A and ET_B receptor agonist) and sarafotoxin 6c (S6c; ET_B receptor agonist) were studied using a sensitive myograph. The mRNA levels of ET_A and ET_B receptors, and of CAMKII were determined with real-time polymerase chain reaction (PCR) while the protein level was evaluated by immunohistochemistry and western blot.

Results. The mRNA levels of CAMKII and of the ET_B receptor were increased during organ culture but not for ET_A receptor. This effect was abolished by co-incubation with KN93 or U0126. In functional studies, both inhibitors attenuated the S6c and potassium induced contractions, and KN93 decreased the ET_1 induced response. This was confirmed at the protein level by immunohistochemistry where the endothelin receptors were found co-localised with CAMKII. Phosphorylated extracellular signal-regulated kinase p-ERK1/2 and CAMKII was measured by immunohistochemistry and western blot. Incubation of arteries with KN93 decreased the protein levels of p-ERK1/2 and CAMKII while U0126 has no effect on CAMKII.

Conclusion. Our results show that the CAMK II is involved in the endothelin receptor regulation and interacts with the MEK/ERK1/2 pathway, resulting in enhanced receptors expression in rat cerebral arteries.

P10.02

EFFECT OF INTEGRIN-EXTRACELLULAR MATRIX INTERACTIONS ON SMALL ARTERY STRUCTURE AND MECHANICS DURING HYPERTENSION AND AGING

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An age-related increase in systolic blood pressure and similarities in the consequences of hypertension and aging on arterial structure/mechanics have been reported, particularly in larger arteries. As the structural/mechanical properties of smaller resistance vessels in the context of advancing physiological age and the mechanisms underlying these parameters are less well defined, we studied the effect of disintegrin-treatment (kistrin, or echistatin), *ex-vivo*, on mesenteric artery structure and mechanics, using wire-myography, in 8 week-old (young), 15 week-old (adult) and >1 year-old (old) Spontaneously Hypertensive (SHRs) and Wistar-Kyoto rats (WKYs). Vascular integrin α_V and α_5 expression were also quantified. Initial narrowing of the lumen by inward eutrophic remodelling, in arteries from young and adult SHRs, was superseded by expansion of the lumen as a result of outward hypertrophic remodelling in old SHRs. Vascular stiffness was increased in young SHRs

versus WKYs but was similar in adult and old SHRs versus WKYs, despite increased integrin α_V and α_5 expression in these latter two groups. Disintegrin-treatment of arteries did not alter structure or mechanics in SHRs. Aging in WKYs was associated with hypertrophic remodelling of mesenteric arteries which was independent of integrin expression and associated with no change in vascular stiffness. In WKYs at all ages, vascular structure remained unaltered by disintegrin-treatment; however, stiffness was significantly reduced with echistatin-treatment ($P < 0.05$), suggesting that integrin-ECM interactions contribute towards passive stiffness of WKY arteries. Further insight into the influence of integrin-ECM interactions on vascular structure/mechanics will contribute towards a better understanding of hypertension and age-related cardiovascular complications.

P10.03

ENDOTHELIUM-SPECIFIC CALCIUM-ACTIVATED POTASSIUM CHANNELS: TARGETS FOR ALDOSTERONE

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To define the vascular role of the mineralocorticoid receptor (MR) in cardiovascular pathophysiology, we generated a conditional transgenic mouse model with MR overexpression in the endothelium.

We previously described increase sensitivity to several vasoconstrictors in mesenteric arteries such as in aorta of mice over-expressing the MR in the endothelium (MR-EC) in presence of a normal relaxation to vasodilators. *In vivo*, blood pressure (BP) was increased in awaked MR-EC mice, as compared to the controls (CT) (tail-cuff method).

We have investigated the role of the small (SK_{Ca}), and the intermediate (IK_{Ca}) and the big (BK_{Ca}) conductance potassium channels in vascular function. In infra-renal aorta, endothelial SK_{Ca} and IK_{Ca} channel blockade (Apamin 1 μM + TRAM-34 1 μM) unmasked an impaired relaxation to acetylcholine in MR-EC mice as compared to controls. Inhibition of the smooth muscle specific BK_{Ca} channel with Iberiotoxin (0.1 μM) blunted the higher phenylephrin-induced contractile response observed in MR-EC as compared to CT. These pharmacological data suggest that endothelial MR overexpression is associated with an increased activity of EDHF-mediating endothelial Ca-activated K channels and a functional decrease of the smooth-muscle BK_{Ca} channel activity. Western-blot analyses of the protein expression levels of these channel subunits were performed. None of them showed significant modulation, suggesting that channel activity, rather than expression level, is affected in this model.

MR activation specifically in endothelial cells is therefore associated with increased BP and altered vascular reactivity, in absence of renal collecting duct mediated MR effects. This may be related to ion channels remodeling.

P10.04

INFLUENCE OF 1,25(OH) $_2$ D $_3$ (VITAMIN D) ON RESISTANCE ARTERY CONTRACTILITY

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Low serum vitamin D is linked with higher vascular risk. It is unclear how.

Objective: to test whether 1,25(OH)₂D₃ (active vitamin D) modifies contractility of proximal resistance vessels, dose-dependently.

Methods: Male Wistar rat mesenteric arteries were investigated by wire myography. 60 mM High Potassium Physiological Salt Solution (KPSS) was the reference for contraction and relaxation measurements. Noradrenaline (NA) responses were measured after 10 min, 30 min and 3 hours incubation with 1,25(OH)₂D₃, as was endothelial function by Acetylcholine (ACh) response.

Results: KPSS-induced contraction was unaffected by 1,25(OH)₂D₃, but slightly decreased after 3 h incubation in control and 1,25(OH)₂D₃ groups (generally $n = 5$ arteries each). After 10 min NA-induced contraction at 10^{-5} M, a small dose response occurred (controls $192 \pm 22\%$; vitD 10 nM 183% , 100 nM 169%), but after 3 h incubation with 100 nM 1,25(OH)₂D₃, contraction decreased at 3×10^{-6} , and at 10^{-5} M NA to $118.6 \pm 10.3\%$, compared with controls (mean \pm SE: $145.4 \pm 13.9\%$). While differences were individually 'significant' ($p = 0.04$, Wilcoxon test), 2-way ANOVA demonstrated clear vitD ($F_{3,80} = 6.3$, $p = 0.001$) and NA effects ($F_{4,80} p < 0.000$), without interaction. ACh-induced relaxation (at 10^{-9} to 10^{-5} M) after 30 min incubation was not enhanced by any 1,25(OH)₂D₃ dose. After 3 h, higher concentration ACh (10^{-6} , 10^{-5} M) induced constriction. Paradoxically, 100 nM 1,25(OH)₂D₃ marginally increased contractions ($105.2 \pm 4.8\%$; control $91.7 \pm 4.7\%$), not individually 'significant' but by 2-way Anova, both vitD & ACh dose effects were ($F_{3,78} = 6.6$, $p < 0.001$).

Conclusion: To our knowledge, these are the first vitD experiments on proximal resistance vessels. 100 nM vitamin D may decrease NA-induced contraction but paradoxical endothelial effects may underlie its variable in-vivo actions.

P10.05

REDUCED MOLECULAR FLEXIBILITY IN THE LARGE ARTERIES OF DIABETIC RATS

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In Type 1 and 2 diabetes tissue stiffening is evident from measurements of the gross mechanical properties of the vasculature. In general, pathological glycosylation of extracellular matrix proteins may play an important role in increasing stiffness in diabetic patients. However, the effects of diabetes on individual elastic fibre components remain poorly defined.

Fibrillin microfibrils, a key elastic fibre component, have a 'beads-on-a-string' structure with a periodicity of approximately 56 nm. We tested for possible disruption due to diabetes in fibrillin microfibrils isolated from rat aorta. Diabetes was induced in 250 g adult Wistar rats by streptozotocin (STZ) injection (55 mg/kg) and were sacrificed 8 weeks later along with age-matched controls. At sacrifice, the STZ-treated rats had severe hyperglycaemia (± 28 mmol/l). Fibrillin microfibrils were isolated and purified by well-established bacterial collagenase digestion and size-exclusion chromatography prior to imaging with atomic force microscopy.

Initial experiments show that fibrillin microfibril periodicity is reduced following STZ treatment; 52.0 ± 0.4 nm (STZ) vs 56.9 ± 0.4 nm (Control), $n = 600$ periodicity measurements, 2 animals per group, ($p < 0.01$). In young, healthy tissues the structure of fibrillin microfibrils is stabilised by both intra- and inter-chain disulphide bonds and by transglutaminase cross-links which permit reversible extension *in vivo*. These observations suggest that the formation of pathological cross-links may limit microfibril elasticity and hence play an important role in increasing the stiffness of the diabetic vasculature.

P10.06

ABNORMAL VASCULAR PROGRAMMING OF ACID ARACHIDONIC METABOLISM COULD EXPLAIN HYPERTENSION IN RATS EXPOSED IN UTERO TO MATERNAL DIABETES

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Epidemiologic studies have clearly demonstrated that cardiovascular risk is not only determined by conventional risk factors of importance in adult life, but also by early life events resulting of re-settings of key physiological functions. In our model of rats exposed *in utero* to maternal diabetes, we previously identified a specific gene expression profile of the thoracic aorta at a pre-hypertensive stage (3 months) in favour of vasoconstriction, which could explain the development of hypertension in the adult offsprings. We found an increase of CYP4f2 (however we failed to confirm its up-regulation at the protein level),

and a decrease by 50 percent of the prostacyclin (IP) receptor at messenger and protein levels in aorta of rats exposed to maternal diabetes (DMO) compared to rats from control mothers (CMO). We demonstrated the functional implication of this down-expression of the IP receptor in a pharmacological study using a prostacyclin analogue: Iloprost (iv, 4 ng/kg/ml). Indeed, we showed that, even before the onset of hypertension, SBP reduction in response to Iloprost was attenuated in DMO rats (-10.7% vs -21.3% in CMO, $p < 0.05$). In parallel, we studied vascular reactivity and myogenic response of carotid and mesenteric arteries of 18-months-old CMO and DMO. At this later stage, we found similar results, i.e. vasodilation in response to Beraprost was reduced in DMO, and myogenic response was increased.

In this study, we clearly demonstrated a fetal programming of the vessels, which could explain the development of hypertension and a re-setting of physiological functions in adult rats exposed to maternal diabetes.

P10.07

A PPG MEASUREMENT SETUP AND PULSE WAVE ANALYSIS FOR ARTERIAL STIFFNESS

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The light energy absorption of whole blood in the visible and infrared range is partly caused by the oxidized and reduced haemoglobin. The measurement principle can be applied for photoplethysmography (PPG). Our PPG measures the blood flux in human vessels with means of red and infrared light absorption. The absorption of light varies with the oxygen concentration and amount of blood in vessels. The PPG device has phase sensitive detection electronics which proved to be a good solution for the measurement of small absorption signals simultaneously at two different wavelengths, 660 and 940 nm. In practice, the PPG waveforms, called pulse waves, can be rapidly and simply acquired by a PIN photodiode which measures the transmission of red and infra-red LED light through the forefinger and the second toe simultaneously. The waveforms are characteristics for the young person but different for the elderly person. The four template waveforms are in the consideration for waveform analysis and we get the accurate results enough. The PPG amplitude can increase and decrease caused by the autonomic fluctuation. In the wave analysis, the first wave is called a percussion wave, the second is called tidal wave, and the third is a dicrotic. The PPG measurements may provide cheap, simple and accurate methods of diagnosing arterial and, especially vascular diseases. Moreover, further development of the theoretical model that correlates the waveform of the detected finger tip wave caused by heartbeat oscillations and the hemodynamic parameters could improve the accuracy of the method and potentially lead to a better quantification of the measured parameters used for arterial stiffness.

P10.08

PULMONARY ARTERY CALCIFICATION IN RACEHORSES

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Vascular calcification (VC) has been sporadically reported in horses, but little is known regarding cause, pathogenesis and clinical significance.

We hypothesized that in horses, structural and molecular changes may occur during VC that are comparable to human and mouse models. We surveyed Thoroughbred and Standardbred racehorses ($n = 101$) for the prevalence, distribution and severity of VC. Histopathological, ultrastructural imaging and energy dispersive X-ray elemental analyses were used to examine the lesions. Immunohistochemistry for cell markers (smooth muscle α -actin, SM22 α and Sox9) was performed in selected samples from control ($n = 10$), mildly ($n = 10$), and severely ($n = 10$) calcified arteries.

Results showed that calcification of the tunica media of mainly the pulmonary artery branches, was present in 82% of horses, and both breeds and genders were similarly affected. Lesions appeared as white-to-yellowish, hard, gritty plaques of variable size. Microscopically, elastic fibers were thin, fragmented and calcified, and surrounded by dense collagen matrix, as described for Mönckeberg sclerosis. Elemental analysis of the calcified areas was consistent with hydroxyapatite mineral.

No immunoreactivity for the smooth muscle cell markers, smooth muscle α -actin and SM22 α was observed in cells found at the calcification site. Many of these cells had a chondrocytic phenotype appearance and showed immunoreactivity for Sox9, a chondrocyte marker.

Arterial calcification in horses share histopathological features with arterial medial calcification in humans and may result in similar physiological abnormalities such as vascular stiffness. The occurrence of VC in young racing horses indicates the need to investigate its pathogenesis and potential clinical implications.