P02-P13

Altered endothelial function in the thoracic aorta of LOX-1 overexpressing mice

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The lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is the major receptor for cellular uptake of oxidized LDL-cholesterol (oxLDL) from the blood. This receptor is primarily expressed on endothelial and smooth muscle cells. Several in vitro and in vivo studies showed an up-regulation of the LOX-1 receptor under atherosclerotic conditions.
Aim of this study was, to analyze the impact of endothelial LOX-1 receptor overexpression on vascular function in the thoracic aorta and on metabolic parameters. LOX-1 transgenic mice (LOX-1tg) were fed for 20 weeks with a standard diet (11 % fat, cholesterol free), or a high-fat diet (39 % fat, 2 % cholesterol). C57BL/6 (wild type; WT) mice served as controls. We found no difference in body weight between the WT and LOX-1tg mice after 20 weeks on standard or high-fat diet. Vascular function was analyzed in the thoracic aorta using a Mulvany myograph. Compared to WT, LOX-1tg with control diet had an impaired endothelium-dependent relaxation, but showed no further impairment after high-fat diet. The endothelium-dependent relaxation was completely blocked by the NO synthase inhibitor nitro-L-arginine methylester (L-NAME). In WT and LOX-1tg vessels, endothelium-independent relaxation was not altered on standard or high-fat diet. Organ weight of heart, kidney and liver in LOX-1tg after high-fat diet showed no differences compared to WT on high-fat diet. The absolute amount of white epididymal adipose tissue was significantly increased in LOX-1tg compared to WT. This was further increased after high-fat diet. Therefore, we analyzed the mRNA expression of leptin, adiponectin, resistin and TNF-α in white epididymal adipose tissue by RT-PCR. Leptin and TNF-α mRNA expression was increased in LOX-1tg mice on high-fat diet, compared to WT. Adiponectin and resistin mRNA expression was unchanged.
We conclude that LOX-1 overexpression causes functional changes in the thoracic aorta which might be influenced by adipocytokines of the white adipose tissue, especially by TNF-α or leptin.

P02-P14

Angiotensin II is an endogenous neurotransmitter for rat and human mesenteric resistance blood vessels

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Angiotensin II (Ang II) is one of the most potent vasoconstrictors. We document here the innervation of rat and human mesenteric resistance arteries (MRA) by angiotensinergic neurons of the rat and human sympathetic coeliac ganglia. Angiotensinogen (Ang-N)-mRNA and angiotensin converting enzyme-mRNA but no renin-mRNA were detected by using quantitative real time polymerase chain reaction in total RNA extracts of rat coeliac ganglia. In the same extracts, cathepsin D-mRNA was detected: This protease also cleaves Ang I from Ang-N and could therefore account for the generation of neuronal Ang peptides in the absence of renin. In situ hybridization confirmed the presence of Ang-N-mRNA in the cytoplasm of rat coeliac ganglia. By using solid-phase extraction, high performance liquid chromatography and subsequent radioimmunoassay, Ang II and its metabolites were detected in rat and also in human coeliac ganglia. Immunoreactivity for Ang II was demonstrated in rat and human coeliac ganglia neurons and their projections innervating MRA. In addition, segmental angiotensinergic innervation of MRA was also observed. By means of confocal laser scanning microscopy we were able to demonstrate the presence of angiotensinergic synapses en passant along side of vascular smooth muscle cells. Our findings could indicate that Ang II is synthesized inside the neurons of sympathetic coeliac ganglia and may act as an endogenous neurotransmitter locally in MRA.

P02-P15

The Duffy antigen receptor for chemokines transports chemokines and supports their promigratory activity

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Chemokines exert their biological role by binding to G protein-coupled receptors (GPCRs). Besides classical GPCRs chemokines interact with „silent" seven transmembrane receptors, which internalize chemokines without conventional signaling, hence their name interceptors (internalizing receptors). Duffy antigen receptor for chemokines (DARC) is an interceptor for inflammatory chemokines expressed on vascular endothelial cells. These cells present chemokines to rolling leukocytes in order to induce their form adhesion and subsequent extravasation. We used DARC-transfected epithelial MDCK cells and DARC-transfected human umbilical vein endothelial cells (HUVECs) grown on transwell inserts to investigate role of DARC in chemokine transport, its contribution to chemokine surface retention as well as its influence in chemokine-induced leukocyte migration and extravasation. We found, that DARC transported intact inflammatory chemokine CCL2 from basolateral to apical side in intracellular vesicles and immobilized the chemokine on the apical cellular surface. DARC it selves accumulated on the apical side of the cellular monolayer in microvillus extensions during the transport. Functional studies clarified role of DARC in chemokine induced transmigration of leukocytes. Presence of DARC significantly increased the number of transmigrated monocytes across the cellular monolayer. In vivo overexpression of DARC on endothelial cells in DARC transgenic mice (mDARCtg) resulted in enhanced leukocyte extravasation into the site of chemokine application and in augmented delayed-type hypersensitivity reaction compared to wild type (WT) mice.

P02-P16

PAF causes vascular barrier failure by acid sphingomyelinase- and ceramide-dependent activation of endothelial TRPC channels

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Introduction
Platelet activating factor (PAF) plays an important role in the pathogenesis of acute lung injury (ALI). Recently, PAF has been shown to increase lung vascular permeability via the formation of ceramide by acid sphingomyelinase (ASM) (Göggel et al., Nat Medicine 2004), yet the underlying cellular mechanisms remain to be elucidated. Here, we tested the effects of the PAF-ASM-ceramide axis on the endothelial calcium concentration ([Ca2+]i), an important regulator of endothelial barrier function.

Methods
Endothelial [Ca2+]i was quantified using our established technique for real time imaging of fura-2 loaded endothelial cells in the isolated perfused rat lung. Edema formation was quantified as weight gain in isolated perfused rat lungs.

Results
PAF (5 nMol/L) increased endothelial [Ca2+]i from 98±3 to 143±7 nMol/L. Imipramin (100 µMol/L), an inhibitor of the acid sphingomyelinase pathway, blocked the PAF-induced [Ca2+]i increase, but had no effect on baseline endothelial [Ca2+]i in the absence of PAF. Addition of exogenous ASM (1 U/mL) or C2-ceramide (50 µMol/L), but not C2-dihydroceramide (50 µMol/L) replicated the endothelial [Ca2+]i increase. The endothelial [Ca2+]i response to PAF was blocked by SKF96365 (30 µMol/L), an inhibitor of canonical transient receptor potential channels (TRPC), but not by ruthenium red (1 µMol/L), a blocker of vanilloid-type transient receptor potential channels (TRPV). Similarly, SKF96365 prevented endothelial [Ca2+]i increases following stimulation with exogenous ASM or C2-ceramide, and markedly attenuated PAF-induced lung edema formation in isolated mouse lungs.

Conclusions
PAF increases lung endothelial [Ca2+]i by a sequence of signaling events which involve activation of ASM, ceramide formation, and activation of TRPC but not TRPV channels. This signaling cascade contributes to PAF-induced lung edema formation and may present an important mechanism in vascular barrier failure in ALI.