Contractions to endogenous and exogenous endothelin-1 in segmental renal arteries of the mouse: Up-regulation in obesity

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Endothelin-1 (ET-1) is implicated in cardiovascular risk factors such as obesity, and the endothelin system is prominent in the kidney. In murine arteries, the contractile profile of the peptide is heterogeneous among different preparations, and the renal vascular bed is largely unexplored. Segmental renal arteries branching from the main renal arteries of age-matched lean and 30 week diet-induced obese WT mice were investigated by isometric tension recording in Halpern–Mulvany myographs. Contractions after administration of big endothelin-1 (bET-1) or ET-1 (both 10 pM to 100 nM) were determined in the absence and presence of l-NAME, followed by full concentration–response curves to serotonin (5-HT) or the TP receptor agonist U46619. At the highest concentrations of bET-1 contractions were similar in rings of lean and obese mice in the absence of l-NAME. Inhibition of NO synthesis facilitated responses particularly in obese animals ($n = 6–9$, $P < 0.01$). Exogenous ET-1 contracted potently preparations of all groups starting from 3 nM on; the response to the peptide was augmented by obesity in the absence and presence of l-NAME (each $n = 6–10$, $P < 0.001$). ECE-activity calculated as the ratio of the responses bET/ET-1 was significantly higher in rings from obese mice in the presence of NO at 10 nM ($n = 5–9$, $P < 0.01$) and at 30 nM of the peptides in the presence of l-NAME ($n = 6–9$, $P < 0.05$). Contractions to 5-HT and U46619 were comparable between groups. These experiments demonstrate the high responsiveness of the renal vascular bed to both endogenous and exogenous ET-1, and an increased activity of the endothelin system in obesity, whereas responses to 5-HT2 and TP receptor activation are unaltered.

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ET-1-induced contraction of renal afferent arterioles of Dahl salt-sensitive rats is impaired by targeted modification of a p66 Shc regulatory phosphorylation site

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The adaptor protein p66 Shc, a longevity-associated product of the Shc1 gene, is implicated in the pathogenesis of age-related diseases and regulation of sensitivity to oxidative stress. The ability of p66 Shc to promote age-related diseases requires phosphorylation of serine 36 residue (Ser36). We have shown that endothelin-1 (ET-1), an important regulator of the renal microcirculation, induces p66 Shc Ser36 phosphorylation and mediates protein–protein interactions of p66 Shc in renal cells. As the known p66 Shc-mediated effects are highly dependent on Ser36 phosphorylation, we specifically modified this amino acid by introducing a knock-in substitution of this amino acid in Dahl salt-sensitive (SS) rats. Cell embryos were extracted from SS rats and mRNA encoding two engineered ZFNs targeting portion of Shc1 gene encoding p66 Shc isoform were injected into the embryo along with a plasmid template encoding an Ala36 modification. The double strand break caused by microinjection of ZFNs targeting Shc1 gene stimulated homologous recombination with co-injected template plasmid containing the desired mutation. We have established a breeding colony of rats with the Ser36 to Ala36 (S36A) substitution. The absence of Ser36 phosphorylation in response to ET-1 was confirmed by Western blotting with phosphospecific antibodies. The juxtamedullary vasculature was isolated for study from genetically modified SS rats and an afferent arteriole was monitored continuously by videomicroscopy. After control diameter measurements, responses to ET-1 (0.001–10 nmol/L) were determined in afferent arterioles. Since preglomerular arterioles isolated from S36A rats exhibit an impaired vascular response to ET-1 when compared with their wild type littermates, p66 Shc is important for ET-1-induced vascular responses in renal vessels.

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Blocking endothelin-1 induced multiple drug resistance permits effective inhibition of activation of renal proximal tubules exemplified by the PKC alpha-microRNA15a loop

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In multiple drug resistance (MDR) cellular transport mechanism, such as the MDR-1 and the MRP1-5 proteins, is induced. Cells become resistant by accelerating the efflux of reagents being reabsorbed. This mechanism could be a major component of therapeutic resistance after proximal tubule activation in chronic proteinuric disease. By qRT-PCR, we identified endothelin-1 (ET-1)-inducible MDR-transport proteins in normal (RPTEC) and tumorous (CAKI-1) proximal tubule cells. Verapamil and elacridar, a first and third generation MDR-inhibitor, blocked those transport proteins demonstrated by calcein-AM-assay. Fluorinated calcium was retained best in the cells after elacridar treatment of prestimulated proximal tubule cells and shows a functional impact. To further analyse the effect of therapy after blocking MDR we used a previously (von Brandenstein et al., 2010) described regulatory loop, causally connecting the upregulation of microRNA15a with the downregulation of PKC alpha after ET-1-stimulation in proximal tubule cells. Selegilin, an inducer of PKC alpha, downregulates micoRNA15a levels in ET1-stimulated proximal tubule cells. The therapeutic dose of selegilin could be significantly reduced after pre-treatment with elacridar. We conclude that: i) blocking the MDR-transport system in proximal renal tubules overcomes chemoresistance after ET-1-stimulation; and ii) therapeutic doses of PKC alpha-inducer selegilin counteracting microRNA15a production can be reduced. This approach is a first step towards an effective therapeutic protection of proximal tubules being activated by the endothelin system contributing to proteinuria in chronic renal disease.

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Evidence for extrarenal vascular endothelin-1 in the maintenance of sodium homeostasis

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