Changes in urinary ET-1 excretion in response to increased renal perfusion pressure in the rat

interventions are hampered by the lack of specific and easily detectable markers. We described a regulatory pathway in which nuclear migration of protein kinase C α controls the release of pri-miRNA15a. After endothelin-1 stimulation the migration of PKCα is inhibited, and mature miRNA15a is made. Using qRT-PCR we detect miRNA15a in the urine of adult and pediatric patients with membranous or minimal change nephropathy. By laser-microdissection this miRNA is predominantly located in the proximal tubules. In cell culture, human RPTECs produce the highest miRNA15a levels after ET-1 stimulation. In rats after 5/6 nephrectomy, miRNA15a is increased in the urine. By graded sieving and qRT-PCR, the highest amount of miRNA15a is found in the tubular fraction. Selegiline treatment upregulates PKCα in vitro and in the murine adriamycin model, significantly downregulating ET-1 induced miRNA15a production. Thus measuring urinary miRNA15a levels: i) indicates the regulation of a signal pathway in RPTECs in vivo in proteinuric conditions; ii) allows for the first time to control the effectiveness of a therapy aiming to protect proximal tubules.

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ET-B receptors in podocytes promote diabetic glomerulosclerosis with β-catenin and NFκB activation

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Recently the endothelin (ET) system has emerged as a novel target for the treatment of diabetic nephropathy (DN) with anti-proteinuric actions. However, no evidence for a direct in vivo pathogenic effect of ET-1 on podocytes has been shown. Despite podocyte dysfunction in DN, specific ET-1 signaling in podocytes has not been investigated. This study investigated ET signaling in podocytes during experimental DN. We first demonstrated that the prominent functional ET-1 receptors eliciting rapid calcium transients in podocytes are ETBRs. Mice with a podocyte-specific double deletion of ETAR and ETBR (Pod-ETRKO) were rendered diabetic by streptozotocin injection. Whereas wild-type diabetic mice developed mild DN with microalbuminuria, mesangial matrix expansion and podocyte loss, Pod-ETRKO mice were protected from diabetes induced glomerulosclerosis and podocyte loss. We next found that total β-catenin and phospho- NFκB expressions are strongly reduced in glomeruli from Pod-ETRKO mice. Moreover, ET-1 could directly activate β-catenin and NFκB signaling pathways in freshly isolated glomeruli. This is the first evidence that ET-1 activation drives development of glomerulosclerosis and podocyte loss through direct activation of ETRs in podocytes, and likely, through NFκB and β-catenin pathways. Surprisingly, both at the expression level and the functional level, the ETBR subtype was found to be prominent. Furthermore, these results indicate that activation of the ET-1 pathways selectively in podocytes is involved in pathophysiological crosstalk that influences mesangial architecture and sclerosis.

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Changes in urinary ET-1 excretion in response to increased renal perfusion pressure in the rat

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Urinary ET-1 (UET-1) excretion may result from cellular exposure to shear stress but the relative contributions of renal blood flow and tubular flow are not known. We measured changes in UET-1 in response to changes in renal perfusion pressure (RPP) in the rat in order to identify associations between UET-1, urinary flow rate (UV), urinary sodium excretion rate (UNaV) and RPP. Methods: Seven male Sprague Dawley rats weighing 258 ± 17 g underwent induced pressure natriuresis. Arterial blood pressure was measured directly and urine was collected via tube cystotomy. RPP was initially increased by ligation of both the coeliac and cranial mesenteric arteries and subsequently of the distal aorta. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined by FITC-inulin and para- aminohippurate clearance, respectively. UET-1 was measured by a sandwich ELISA assay (Biomedica). Urinary sodium was measured by sodium-selective electrode analysis. Results: GFR and ERPF did not change significantly when RPP increased from 137 ± 9 to 162 ± 11 mmHg but significant increases in UV (4 ± 1 to 97 ± 33 μl/min/g kw) and UNaV (1 ± 1 to 23 ± 9 μmol/min/g kw) had linear relationships with RPP ($r2 = 0.39$, $p = 0.022$ and $r2 = 0.51$, $p < 0.001$ respectively). UET-1 increased from 11 ± 7 to 42 ± 26 fg/min/g kw ($p = 0.014$) but was not predicted by RPP. UET-1 did, however, strongly correlate with UV ($r = 0.69$, $p = 0.001$) and UNaV ($r = 0.65$, $p = 0.002$). Conclusions: In the rat, UET-1 is more associated with renal tubular flow than renal blood flow at higher RPPs. Funded by Moray Endowment Fund and British Heart Foundation.

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