It has recently been established that a high salt diet leads to sodium storage within the skin of rats and mice. This increase in sodium in the highly vascularized skin results in macrophage infiltration and lymphangiogenesis. While dysfunction in this process has been implicated in salt sensitive hypertension, the mechanisms are poorly understood. Because vascular endothelin-1 (ET-1) is up-regulated in response to a high salt diet or hypertension, and is a potent chemoattractant, we hypothesized that endothelial derived ET-1 mediates infiltration of immune cells into the skin during chronic high salt intake, thereby allowing sodium clearance from the skin and preventing accumulation. Our data indicate that increasing extracellular concentration of human endothelial cells by 40 mOsm with NaCl, similar to what is seen in the interstitial space of rats placed on a high salt diet, leads to a 50% increase in ET-1 production, a mechanism likely mediated by TonEBP. Furthermore, in response to one week of high salt diet, skin Na/water ratio was elevated in vascular endothelial cell ET-1 knockout mice compared to wild type mice (0.112 ± 0.007 vs. 0.096 ± 0.004 mmol/ml). These data suggest a critical role for ET-1 in preventing the accumulation of sodium in the skin during a high salt intake, an emerging mechanism of the body’s ability to buffer blood pressure changes in response to increases in sodium intake.


Endothelin converting enzyme inhibition attenuates early albuminuria and late renal failure in streptozotocin induced diabetic mice
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Background: Recent clinical trials with endothelin receptor antagonist on diabetic nephropathy have been terminated by side effect of fluid retention in spite of their beneficial effect for proteinuria. The alternative approach for interrupting endothelin pathway by endothelin converting enzyme (ECE) inhibition is anticipated. Methods and results: We injected streptozotocin (STZ; 180 mg/kg i.p.) once for generating type 1 diabetes into ten-week old male ECE-1 heterozygous knockout (KO) mice and their wild type (WT) littermates. The analysis for kidney function and morphology has been performed since early stage at 2 months until late stage at ten months after STZ. In early phase, diabetic mice have shown remarkable hyperglycemia, polycyuria, renal hypertrophy, and glomerular hypertrophy at the same degree between WT and KO mice. But ECE-1 inhibition suppressed protein urea and albumin urea (WT vs KO: 1011 ± 387 vs 283 ± 69 μg/day, p < 0.05). Along with time passage until late stage, polycyuria and glomerular hyperfiltration state made the shift to oligourea. ECE-1 inhibition attenuated the progression of renal failure showing preserved GFR (WT vs KO: 238 ± 48 vs 370 ± 59 μl/min) and renal atrophy (WT vs KO: 12.6 ± 0.8 vs 14.8 ± 0.7 mg/mm tibia length). Superoxide in whole kidney quantified lucigenin assay has increased in WT mice and suppressed in KO mice (WT vs KO: 112 ± 10 vs 70 ± 6 cmppm).

Tubular fibrosis by silius red staining has also prevented in KO mice. Conclusion: ECE-1 suppression prevents the progression of renal failure by suppressing glomerular barrier damage, oxidative stress, and tubular fibrosis in diabetic mice.


High salt diet attenuates ET-1 mediated calcium signaling responses in preglomerular smooth muscle cells from WT and ETB receptor-deficient rats
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High salt diet reduces myogenic reactivity in resistance vessels and autoregulatory responses of juxtaglomerular afferent arterioles. High salt diets increase endogenous endothelin levels, and enhance preglomerular ETB receptor expression. Renal autoregulatory responses are modulated by endothelin receptors and endothelin-mediated preglomerular vasoconstriction requires elevation of intracellular calcium concentration. Accordingly, experiments were performed to determine the impact of high dietary salt on the endothelin-mediated calcium signaling responses in freshly isolated preglomerular microvascular smooth muscle cells (PMVSMCs). Kidneys were harvested from wild type (WT) and ETB-deficient (ETBdef) rats fed normal salt (NS, 0.4% NaCl) or high salt (HS, 8% NaCl, 14 days) diets and PMVSMCs were prepared. Calcium signaling responses were studied using fura-2-based photometry. PMVSMCs isolated from WT rats on NS responded to ET-1 (10 nM) with a biphasic increase in intracellular calcium from a baseline of 63 ± 6 nM to a peak response of 983 ± 141 nM (P < 0.05) and in PMVSMCs isolated from WT rats fed HS, intracellular calcium increased from 56 ± 4 nM to 423 ± 95 nM or approximately 38% of the NS response (P < 0.05). In contrast, PMVSMCs isolated from ETBdef rats on NS responded to ET-1 with an increase in intracellular calcium from 49 ± 4 nM to 713 ± 152 nM (P < 0.05) whereas cells from HS ETBdef rats increased intracellular calcium from 52 ± 4 nM to 418 ± 117 nM or approximately 50% of the NS response (P > 0.05). These data demonstrate that ET-1 signals in PMVSMCs with an elevation of intracellular calcium concentration. In addition, these data demonstrate that increasing dietary salt blunts the calcium response to ET-1.


Collecting duct NOS1 knockout mice lack ET-1 mediated inhibition of collecting duct ENaC
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On a normal salt diet (NSD) flow control and principal cell-specific collecting duct, NOS1 knockout (CDNOS1KO) mice display similar blood pressure (BP). On high salt diets, CDNOS1KO mice retain Na with significantly increased BP. Given that ET-1 increases NO in the CD, we hypothesized that CDNOS1KO mice have a dysfunctional

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renal ET pathway. To test this hypothesis, we measured urinary ET-1 excretion, inner medullary (IM) ET receptor (ETR) expression from mice on a NSD or 7 day high salt diet (7DHS) and the response of ET-1 mediated epithelial sodium channel (ENaC) activity in flox and CDNOS1KO mice. ET-1 excretion was similar between NSD flox and CDNOS1KO mice (0.14 ± 0.02 and 0.17 ± 0.06 pg/day, n = 10) and significantly increased similarly after a 7DHS (0.60 ± 0.1 and 0.60 ± 0.08 pg/day). IM ETR expression was similar between the mice on a NSD (~40% ETA, ~60% ETB receptors) and similarly shifted to ~95% ETRB expression on a 7DHS. Basal CD ENAC open probability (Po) was similar (flox: 0.3 ± 0.08 CDNOS1KO: 0.3 ± 0.05). Acute ET-1 treatment significantly reduced ENaC Po from flox mice but not CDNOS1KO mice compared to basal (flox 0.1 ± 0.03 and CDNOS1KO 0.3 ± 0.05, n = 6 animals P < 0.05). In conclusion, CD NOS1 appears not to regulate renal ET-1 production or ETR expression. However, the mechanism of ET-1 inhibition of CD ENaC is via NOS1. We propose that the salt-dependent increase in BP and Na retention observed in CDNOS1KO mice is mediated by the loss of ET signaling in the CD.


Increased of heparanase expression in hypoxic endothelial cells and kidney ischemic-reperfusion injury associates with endothelin-1 elevation and eNOS reduction

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Renal ischemia/reperfusion injury (I/R) is the most frequent cause of acute kidney injury. It had been reported that endothelin-1 deletion from endothelial cells attenuates I/R injury. Heparanase is an enzyme that degrades endothelial surface layer and induces endothelial injury. The association between heparanase and ET-1 in kidney I/R is still unclear. We induced hypoxic condition for 30 min and characterized by myoﬁbroblast formation. ETAR from interstitial cells may induce myoﬁbroblast area, and the capillary number using Sirius Red, α-SMA, and CD31 immunostaining. Double α-SMA and PDGFRβ staining and quantification were done to examine interstitial cell expansion. Renal blood flow was observed and quantified by laser Doppler imaging. Western blot was done to examine α-SMA, PDGFRβ and TGFβ1 expression. Kidney ET-1 system was measured using ELISA and real time PCR. Double α-SMA and ETAR immunostaining was done to elucidate ETAR in myoﬁbroblast cells. We found significantly lower fibrosis, myoﬁbroblast area, and TGFβ1 expression (p < 0.05) in VEETKO mice compared to WT mice. Kidney ET1 and pre-pro ET-1 mRNA levels increased after UUO, however were significantly lower in VEETKO mice. VEETKO mice also had signiﬁcantly lower interstitial cell expansion and myoﬁbroblast area compared to WT mice. EC derived ET-1 deletion also improved renal blood flow and capillary number (p < 0.05) after UUO. We observed ETAR expression in the myoﬁbroblast area and is colocalized with PDGFRβ. EC derived ET-1 deletion attenuates kidney ﬁbrosis via preserving the capillary and reducing interstitial cell expansion and myoﬁbroblast formation. ETAR from interstitial cells may induce proliferation and myoﬁbroblast formation. Targeting the ET-1 and ETAR axes in EC and interstitial cell may give the best approach to treat kidney ﬁbrosis.


Hypoxia stimulates glomerular reactive oxygen species through an endothelin-1/ET-A dependent mechanism

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