Sickle cell nephropathy (SCN) is a chronic manifestation of sickle cell disease (SCD). We previously found that reactive oxygen species (ROS) were elevated in the glomeruli from SCD mice. Importantly, treatment with ABT-627, a selective ET-A receptor antagonist, reduced glomerular ROS production in SCD mice to levels observed in controls. Because SCN is thought to progress secondary to repeated occlusions in the microvasculature, we hypothesized that hypoxia stimulates the production of glomerular ET-1, causing deleterious effects through over-production of ROS. To directly test if ET-1 is increased in response to hypoxia in unaffected mice, we exposed vascular endothelial ET-1 knockout (VEET) mice and floxed controls to normoxia or 3 h of hypoxia (8% O_2). In response to hypoxia, floxed mice had significant increases in glomerular ET-1 mRNA while there was no response to hypoxia in the VEET mice, indicating that hypoxia stimulates endothelial-derived glomerular ET-1 (floxed hypoxia: 2.1 ± 0.2, floxed normoxia: 1.1 ± 0.2, VEET hypoxia: 0.7 ± 0.1, VEET normoxia: 0.8 ± 0.3 fold change, $p < 0.01$, $n = 6$/group). To determine the influence of chronically elevated ET-1 on glomerular ROS production, C57BL/6J mice were treated with saline or ET-1 (2 weeks @2 pg/kg/day) via a miniosmotic pump. Mice treated with ET-1 demonstrated significant increases in stimulated ROS production compared to saline controls (5452 ± 655 vs. 2177 ± 359 luminescence/protein∗min, $p < 0.01$, $n = 5$–6). These data reveal that hypoxia leads to upregulation of endothelial-derived glomerular ET-1 and that chronic elevations in ET-1, similar to what is seen in SCD, increase ROS production in the glomeruli. Taken together, these data identify a novel mechanism by which hypoxia stimulates the upregulation of ET-1 in glomerular endothelial cells and promotes glomerular ROS production.

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Combined endothelin A receptor and renin–angiotensin system blockade is superior to isolated renin–angiotensin system blockade against the progression of renal damage in 5/6 nephrectomized Ren-2 transgenic hypertensive rats

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Hypertension plays a critical role in the progression of chronic kidney disease (CKD). Recent studies have shown that besides the inappropriately activated renin–angiotensin system (RAS), enhanced intrarenal activity of the endothelin (ET) system via activation of ET receptor type A (ETA) contributes to the pathophysiology of hypertension and progression of CKD. We therefore evaluated whether addition of selective ETA receptor blockade to the standard RAS blockade will exhibit additional beneficial effects on the progression of CKD. Ren-2 transgenic rats (TGR) underwent 5/6 renal ablation (5/6 NX) serving as model of CKD. A combination of angiotensin-converting enzyme inhibitor (trandolapril, 6 mg/l drinking water) and angiotensin II receptor blocker (losartan, 100 mg/l drinking water) was used. ETA receptor blocker (atrasentan, 5 $mg \cdot kg^{-1} \cdot day^{-1}$) was employed with the combination of RAS blockade. The follow-up period was 44 weeks after 5/6 NX. The following parameters were evaluated: survival rate, systolic blood pressure (SBP), proteinuria and renal glomerular damage. Both therapeutical regimes improved survival rate, however the efficiency of isolated RAS blockade considerably decreased at 36 weeks after 5/6 NX (final survival rate was 65%). The combined RAS and ETA receptor blockade exhibited a final survival rate of 91%, which was significantly better as compared with isolated RAS inhibition, even if there were no significant differences in SBP among the experimental groups. In addition, the RAS and ETA receptor blockade further reduced proteinuria and renal glomerular damage. Our data show that a combined RAS and ETA receptor blockade exhibited additional beneficial effects on the progression of CKD in 5/6 NX TGR as compared with isolated RAS inhibition.

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Endothelial cell-derived ET-1 contributes to the severity of septic kidney injury

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Septic acute kidney injury (AKI) remains associated with high mortality rate, partially, because of the poor understanding of its (patho)physiology. Endothelin-1 (ET-1), which is produced and is secreted from vascular endothelium, plays important roles on renal hemodynamics via autocrine/paracrine and, possibly, hormonal mechanisms. Previous studies have demonstrated that either selective or non-selective endothelin antagonists attenuate the hemodynamic changes in experimental sepsis models. Thus, we investigated the role of endothelial cell-derived ET-1 on the severity of lipopolysaccharide (LPS)-induced AKI by using endothelial cell-specific ET-1 knock-out mice (VEETKO). The renal microcirculation dynamics was observed by intravital 2-photon laser microscopy. LPS (from *Escherichia coli* O55:B5, 5 mg/kg, i.p.) increased leukocyte attachment in the capillary walls, whereas there was maintained plasma flow in the peritubular capillaries in VEETKO. The urine flow rate was reduced to half in VEETKO compared with that in normal (LPS-untreated) C57B6 mice, while the blood urea nitrogen level was still at normal level in VEETKO (22.8 ± 0.2 mg/dL vs. 52.9 ± 12.7 mg/dL in C57B6) at 24 h after LPS injection. Fluid resuscitation (1.5 mL of saline, s.c., at 6 and 14 h after LPS) normalized the LPS-induced increase in leukocyte attachment and the decrease in urine flow rate in VEETKO, but the effects were partial in C57B6 mice. These results suggest that VEETKO kidney is less sensitive against endotoxemia than the kidney of C57B6 mice, and that leukocyte attachment and reduction of urine flow in VEETKO may be due to the decrease in blood pressure by endotoxemia in combination with the lack of ET-1.

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Renal phenotype of type 1 diabetic endothelial cell derived ET-1 deficient mice

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