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Arginine uptake is attenuated through modulation of cationic amino-acid transporter-1, in uremic rats

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Endothelial cell dysfunction (ECD) is a common feature of chronic renal failure (CRF). Defective nitric oxide (NO) generation due to decreased endothelial NO synthase (eNOS) activity is a crucial parameter characterizing ECD. L-arginine is the sole precursor for NO biosynthesis. Among several transporters that mediate L-arginine uptake, cationic amino-acid transporter-1 (CAT-1) acts as the specific arginine transporter for eNOS. Our hypothesis implies that CAT-1 is a major determinant of eNOS activity in CRF. We studied glomerular and aortic arginine uptake, CAT-1, and CAT-2 messenger ribonucleic acid (mRNA) expression, and CAT-1 protein in: (a) rats 6 weeks following 5/6 nephrectomy (CRF), (b) sham-operated animals, and (c) rats with CRF treated orally with either atorvastatin or arginine in drinking water (modalities which have been shown to enhance eNOS activity and improve endothelial function). Both glomerular and aortic arginine transport were significantly decreased in CRF. Treatment with either arginine or atorvastatin abolished the decrease in arginine uptake in CRF rats. Using reverse transcriptase-polymerase chain reaction and Northern blotting, we found a significant increase in glomerular and aortic CAT-1 mRNA expression in CRF. Western blotting revealed that CAT-1 protein was decreased in CRF, but remained intact following arginine and atorvastatin administration. Renal and systemic arginine uptake is attenuated in CRF, through modulation of CAT-1 protein. These findings provide a possible novel mechanism to eNOS inactivation and endothelial dysfunction in uremia.

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Endothelial cell dysfunction (ECD) is a common precursor and denominator of patients with chronic renal failure (CRF). This syndrome is currently interpreted as encompassing disturbances in several functions: the barrier function of vascular endothelium, its impaired antithrombogenic properties, blunted angiogenic capacity, inappropriate regulation of vascular smooth muscle tonicity, proliferative capacity, and migratory properties.[1] Accumulated evidence suggests that many of these abnormalities are linked to the capacity of the constitutive, Ca^{2+}/calmodulin-sensitive nitric oxide (NO) synthase (NOS) to generate adequate quantities of NO.[2] Indeed, reduction in NO generation has been reported in both experimental CRF and peritoneal, and hemodialysis patients.[3–6] Abnormal function of the endothelial NOS (eNOS) system that results in ECD includes: decreased eNOS expression, alteration of NO signaling, destruction of NO by reactive oxygen species, and impaired availability of cofactors.[7] Our interest in this area is focused on the role of intracellular arginine availability in governing eNOS activity, thus affecting endothelial function. *In vitro* studies performed by Baylis *et al.*[8,9] suggest that arginine uptake is decreased in renal failure. Moreover, they have shown that serum of uremic patients has the capacity to inhibit arginine uptake by cultured endothelial cells, an effect attributed to urea uptake by endothelial cells via urea transporter type B.

Among several transport systems that mediate L-arginine uptake (y^+, $b^{0,+}$, $B^{0,+}$, and y^+ L), system y^+ is widely expressed and considered to be a major arginine transporter in most tissues and cells. Encoded by cationic amino-acid transporters (CAT)-1, CAT-2, and CAT-3, system y^+ is characterized by high affinity for cationic amino acids, sodium independence, and stimulation of transport by substrate on the opposite (*trans*) side of the membrane.[10,11] Accumulated evidence from our laboratory and others suggest that each transporter has affinity to a specific NOS isoform. We have previously shown that arginine uptake by tubules or glomeruli harvested from rats subjected to ischemia and reperfusion or to sepsis, two experimental models characterized by activation of the inducible NOS, exhibit augmented arginine uptake, associated with

upregulation of CAT-2.[12,13] In contrast, we have also suggested that increased arginine uptake through upregulation of CAT-1 may contribute to the pathogenesis of diabetic hyperfiltration, via activation of eNOS.[14] Schafer *et al.*[15] have shown that suppression of the endothelium-mediated microvascular vasodilation by dexamethasone involves downregulation of eNOS and CAT-1. Moreover, CAT-1 and eNOS were found to be colocalized in a caveolar complex;[16] therefore, this complex has been suggested to serve as a mechanism for channeling of newly acquired extracellular arginine to eNOS, for NO synthesis. In the aggregate, it is believed that arginine is delivered to eNOS predominantly by CAT-1.

Experiments described herein were designed to determine whether glomerular and aortic arginine uptake are altered in an *in vivo* model of CRF and to elucidate whether these changes are related to the specific eNOS arginine transporter CAT-1 mRNA expression and protein abundance in these tissues. In addition, we studied the beneficial effects of arginine and statins, two modalities previously shown to improve endothelial function, on the above parameters, aiming to link our findings to ECD in CRF.

RESULTS

Effects of atorvastatin and L-arginine on creatinine clearance

To consolidate previous observations and validate the therapeutic efficiency of arginine and atorvastatin in CRF, creatinine clearance (CCr) was measured in all experimental groups (Figure 1). CRF resulted in a significant decrease in CCr (0.23 ± 0.04 vs 0.55 ± 0.05 ml/min/100 g body weight (BW), $P < 0.01$). Administration of L-arginine or atorvastatin significantly attenuated the decrease in CCr in CRF animals (0.47 ± 0.09 and 0.42 ± 0.08 ml/min/100 g BW, respectively).

Aortic and glomerular arginine transport

Initially, we wished to explore the possibility that CRF affects the characteristics of y^+ system, the predominant arginine uptake system.

Figure 2a and b demonstrates that, in aortic rings and glomeruli harvested from animals subjected to 5/6 nephrectomy, arginine transport system remained sodium independent. Excess concentration of lysine strongly inhibited L-arginine uptake, while the neutral amino acid methionine was found to be a poor inhibitor. In addition, we characterized the kinetics of L-arginine transport in both tissues by measuring the saturable uptake of L-arginine (0–1 mM). The plots of L-arginine uptake as a function of extracellular L-arginine concentration are shown in Figure 3a and b. A high-affinity transporter was found to be present with a K_m of 120 and 125 μM in aortic rings and glomeruli, respectively. These data establish that, in glomeruli and aortic rings from rats with CRF, system y^+ remains the predominant arginine transport system, with kinetic properties that resemble those of CAT-1 and CAT-2.

The next set of experiments was designed to explore a possible effect of CRF on y^+ system and the impact of agents that have been shown to improve endothelial function via modulation of the NO system, namely arginine and statins. We have chosen to perform these experiments in both aortic and renal tissues in order to explore differences between renal and systemic circulation. When compared to normal rats, CRF induced a significant ($\sim$50%) decrease in both aortic and glomerular arginine uptake. Treatment of CRF rats with either arginine or atorvastatin completely abolished this effect (Figure 4a and b).

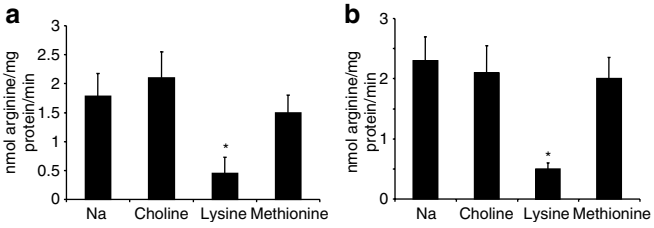

Figure 2 | Sodium independence and *cis*-inhibition by lysine of (a) aortic and (b) glomerular arginine uptake in CRF rats. Uptake of radiolabeled arginine (L-[^{3}H]arginine) by freshly harvested glomeruli and aortic rings from CRF animals, in the presence of either 140 mM sodium chloride or 140 mM choline chloride and either lysine or methionine 10 mM added to the transport solution. Data are presented as the mean $\pm$ s.e.m. of four different experiments. *$P < 0.05$ vs control.

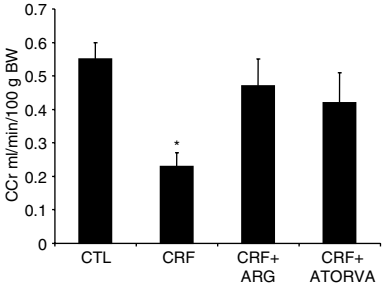

Figure 1 | Effect of atorvastatin and arginine on GFR indexed by CCr in CRF. Results shown are mean $\pm$ s.e.m. *$P < 0.05$ vs controls ($n = 5$). CTL: controls, CRF: chronic renal failure, ARG: L-arginine, ATORVA: atorvastatin.

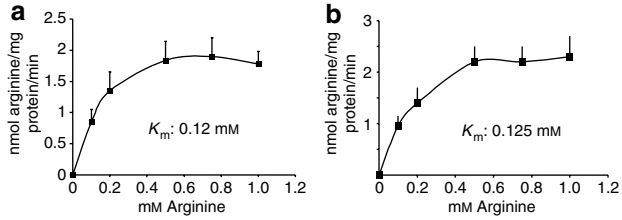

Figure 3 | Concentration dependence of L-arginine uptake by (a) aortic rings and (b) glomeruli from CRF rats. Uptake of [^{3}H]arginine was measured, for 1 min in freshly harvested glomeruli and aortic rings from CRF rats over a range of concentrations (0–1 mM). Data are presented as the mean $\pm$ s.e.m. of four different experiments.