Carbonic anhydrase inhibition - a novel therapeutic strategy for renal disease

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Diabetic nephropathy is characterised by initial renal hypertrophy followed by progressive renal fibrosis eventually leading to end-stage renal disease. Research has focused on fibrotic processes and downstream effects of extracellular matrix accumulation, however, metabolic stress and hemodynamic factors (such as increased Angiotensin II (AngII) promote proximal tubular epithelial cell (PTEC) dysfunction prior to fibrosis. PTEC hypertrophy in diabetic nephropathy is associated with increases in the filtered load of Na^+. Primary transport functions of the proximal tubule are linked, in that availability of bicarbonate and activity of carbonic anhydrases (CAs) play a rate-limiting role by providing substrate for a number of these transporters. The cytosolic carbonic anhydrase (CAII) has been recently found to bind directly to membrane transporters to form so-called metabolons. Here the co-localisation of CAII with the transporters serves to maximise the HCO_3^- gradient for optimal transporter function.

This study seeks to delineate the hypertrophic effects from the fibrotic effects of AngII with a focus on the role of CA2 and whether modulation of CAs in the proximal tubule could have beneficial effects in preserving PTEC function under pathological conditions. The roles of CAs were investigated *in vitro* (HK-2 and OK cell lines) and *in vivo* (Ren2 rat model). Effects on CAII/IV expression were examined by Western blot and immunohistochemistry. Albumin uptake was measured using a fluorescent probe. The roles of CAs in AngII-induced PTEC hypertrophy and altered protein reabsorption were assessed using the CA inhibitors acetazolamide (ACZ) and ethoxyzolamide (ETZ). TGF-1 production was measured by ELISA.

Exposure of PTECs to AngII induced a significant hypertrophic response that was inhibited by the membrane permeable CA inhibitor ETZ but not membrane impermeant ACZ. This demonstrated that this effect was likely mediated by CAII. PTECs exposed to high glucose, AngII and TGF-β1 displayed impairment of normal protein reabsorption. This effect was significantly decreased in the presence of ETZ while the normal basal rate of protein reabsorption was unaffected. Under these conditions, protein expression of CAII was upregulated in PTECs. ETZ also significantly reduced high glucose-induced TGF-1 production in PTECs. In a Ren2, streptozotocin-induced diabetic rat model, elevated levels of CAII were detected both on Western blot and in the proximal tubules by immunohistochemistry.

These data clearly show that CAII plays a key role in mediating the hypertrophic response in PTEC and its levels are altered in diabetic nephropathy. Strategic inhibition of CAII may be of benefit in slowing the early onset diabetic nephropathy.