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Renal purinoceptors and tubular function

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In recent years it has become increasingly evident that purinoceptors, responsive to extracellular ATP and other nucleotides/nucleosides, are widely expressed in the kidney, both in the vasculature and the tubular epithelium. However, what they are doing there is far from clear. The present contribution will focus on P2 purinoceptors in the renal tubule; it will not consider vascular purinoceptors or tubular adenosine (P1) receptors. P2 receptors are of two broad types: P2X (mammalian sub-types 1–7), which are ionotropic, and P2Y (mammalian sub-types 1, 2, 4, 6, 11–13), which are metabotropic (G-coupled) receptors. Unfortunately, 'mapping' these sub-types along the nephron and identifying their actions has not been straightforward. Problems include species differences, the use of cell lines and cultures rather than native tissue, and poor specificity of agonists and antagonists.

The proximal tubule expresses mRNA for $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors (Bailey *et al.* 2000, 2001*a*). Pharmacological characterisation (from the effects of basolaterally applied agonists on intracellular Ca^{2+} transients) suggests that the dominant sub-type in native tissue is $P2Y_1$, although there is functional evidence also for $P2Y_2$ and/or $P2Y_4$ and $P2Y_6$ receptors. Thus far, apical P2Y receptors have been identified only in cell lines. $P2X_4$ and $P2X_5$ mRNAs have been found in primary cultures of proximal tubule, but the situation in native tissue is unclear. Although a few unrelated actions of ATP on proximal tubules have been reported (e.g. stimulation of gluconeogenesis, attenuation of PTH-induced inhibition of phosphate transport), the physiological role, if any, of P2 receptors in this segment remains speculative.

Purinoceptor distribution in the loop of Henle is not fully characterised, but P2Y receptor mRNA expression has been identified in rat thin descending ($P2Y_1$, $P2Y_2$, $P2Y_6$), thin ascending ($P2Y_1$, $P2Y_2$, $P2Y_4$) and thick ascending ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$) limbs (Bailey *et al.* 2000, 2001*a*). However, functional evidence is inconsistent: basolateral application of agonists in the rat elicits intracellular Ca^{2+} transients in thin, but not thick, limbs. To date, no information relating to apical P2 receptors in loop segments has been published, and no compelling evidence exists for nucleotide-induced physiological effects in the loop.

A somewhat clearer picture is beginning to emerge with respect to the distal nephron. There is evidence for $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2X_5$ and $P2X_6$ receptors in distal nephron segments, particularly the collecting duct – where some purinoceptors are located both basolaterally and apically (Schwiebert & Kishore, 2001). *In vitro* studies in perfused collecting ducts have shown that ATP applied variously to the basolateral or apical membrane can elicit intracellular Ca^{2+} transients and inhibit ENaC-mediated Na^+ reabsorption (Lehrmann *et al.* 2002). Since *in vitro* findings may be viewed with some scepticism, we have recently examined the effect of luminal stimulation of P2 receptors in the intact collecting duct *in vivo*. We found that in rats on a normal diet, intraluminal application of the ATP analogue ATPγS had no effect on the urinary recovery of ^{22}Na that had been injected into the late distal tubule (Bailey *et al.* 2001*b*), whereas in rats on a low-Na^+ diet, in which baseline ENaC-mediated Na^+ reabsorption was enhanced, ATPγS caused a significant increase in ^{22}Na recovery (Shirley *et al.* 2001).

Finally, the possible role of purinoceptors in the pathogenesis of autosomal dominant polycystic kidney disease (ADPKD) deserves mention. Cyst fluid contains high concentrations of ATP (Wilson *et al.* 1999), and ATP can stimulate Cl^- secretion in models of ADPKD (Schwiebert *et al.* 2002), suggesting that ATP plays a key role in cyst enlargement in this condition.

In summary, although investigations of purinoceptor function are hampered by the relative bluntness of the tools currently available, the release of nucleotides from tubular cells, together with the existence of receptors for them, argues for an autocrine/paracrine action. Only when sharper tools are brought to bear on the problem will the full contribution of renal purinoceptors to the control of Na^+ excretion be revealed.

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Role of endothelium in blood presure regulation

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Neural control of renal tubular function

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Interactions between different mechanisms in the regulation of renal function

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Post-transcriptional control of renin synthesis: identification of proteins interacting with renin mRNA 3′-untranslated region

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Stabilization and correct localization of mRNA are important features of renin synthesis. To elucidate the molecular basis of

posttranscriptional control of renin synthesis, we analysed the interaction of human prepro-renin (hREN) mRNA 3′-untranslated region (3′UTR) with proteins of renin synthesizing Calu-6 cells.

To identify hREN mRNA binding proteins, electromobility shift assays, UV cross-linking and RNA-affinity chromatography with subsequent MALDI-TOF-MS were performed. A sequence alignment revealed CU-rich clusters in the 3′-UTR of hREN mRNA. These are highly conserved across species. Moreover, the sequences resemble the 3′-UTR control element LOX-DICE (found in lipoxygenase mRNA and other mRNAs) and the α-stability complex binding site of globin mRNAs. Six proteins were unambigously identified as hREN mRNA 3′-UTR binding proteins: hnRNP E1 (synonyms α-CP or PCBP), hnRNP K, dynamin, nucleolin, YB-1 and MINT-homologous protein. These proteins contain various RNA-binding motifs, and most have been described in the context of mRNA binding and mRNA stabilization. Four proteins, for which antibodies were available, were verified by immunological methods (dynamin, nucleolin, hnRNP E1, YB-1). All immunologically detectable hREN mRNA binding proteins were found not only in free form in the cytoplasm, but also in hREN mRNA containing mRNP complexes (polysomes and postpolysomal free mRNP particles). Four of the six proteins (hnRNPs E1 and K, nucleolin and YB-1) have been identified in other mRNP complexes alone or in combination as crucial components of posttranscriptional control of gene expression, in particular in mRNA stabilization.

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Role of superoxide and nitric oxide in modulating the renal actions of angiotensin II

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Angiotensin II (ANG II) produces renal vasoconstriction and increases sodium tubular reabsorption by acting on AT1 receptors and raising intracellular calcium. However, there is evidence indicating that some of the physiological effects of angiotensin II may be partly mediated by increasing NAD(P)H oxidase-derived vascular superoxide (O_2^-) production. Nitric oxide (NO) is an important regulator of renal function. Because the free radical O_2^- reacts with NO, producing peroxynitrite and decreasing its bioavailability, in the present study the role of the interactions between O_2^- and NO in mediating the renal effects of ANG II were evaluated both *in vivo* and *in vitro*.

An intrarenal infusion of ANG II (16 and 160 pmol kg^{-1} min^{-1}) reduced renal blood flow (RBF) by −19 and −36 % , glomerular filtration rate (GFR) by −18 and −35%, and also water (*UV*, by −32 and −11 %) and sodium excretion (U_{Na}, by −29 and −21 %). ANG II also reduced renal cortical NO activity (measured electrochemically) by −9 and −23 %, and lowered the urinary excretion of nitrites/nitrates (U_{NOx}) by −12 and −24 %). The AT1 receptor antagonist valsartan (160 nmol kg^{-1} min^{-1}) abolished the renal effects of ANG II. Apocynin (10 μmol kg^{-1} min^{-1}), a NADPH inhibitor, increased renal cortical NO concentration (+38 %) and U_{NOx} (+56 %), and also reduced the renal vasoconstriction produced by ANG II. Tempol (8 μmol kg^{-1} min^{-1}), a superoxide dismutase mimetic, also raised renal cortical NO activity (+36 %) and U_{NOx} (+26 %). Pretreatment with apocynin or tempol abolished the effects of ANG II on renal cortical NO concentration, GFR, *UV* and U_{Na}. ANG II (10 nM) increased O_2^- generation (measured *in vitro* as lucigenin chemiluminescence) in renal cortical homogenate by +17 %, while valsartan (10^{-5} M), apocynin (6×10^{-4} M) and tempol (5×10^{-4} M) reduced basal O_2^- generation by −37, −60 and −30 %, respectively. Also, valsartan, apocynin and tempol abolished the effects of ANG II on O_2^- generation. In addition, ANG II (10^{-8} M) raised the generation of peroxynitrite (+21 %) in renal cortical homogenate (measured *in vitro* as luminol chemiluminescence), and this effect was prevented by valsartan, apocynin and tempol. Overall, these data indicate that the renal vascular and excretory effects of ANG II may be modulated by increased NAD(P)H oxidase-derived O_2^- that reduces renal NO bioavailability.

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