Renal purinoceptors and tubular function

D.G. Shirley and M.A. Bailey

Centre for Nephrology and Department of Physiology, Royal Free and University College Medical School, Rowland Hill Street, London NW3 2PF, UK

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 P2Y1, P2Y2, P2Y4 and P2Y6 receptors (Bailey et al. 2000, 2001a). Pharmacological characterisation (from the effects of basolaterally applied agonists on intracellular Ca2+ transients) suggests that the dominant sub-type in native tissue is P2Y1, although there is functional evidence also for P2Y2 and/or P2Y4 and P2Y6 receptors. Thus far, apical P2Y receptors have been identified only in cell lines. P2X and P2X7 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 (P2Y1, P2Y2, P2Y6), thin ascending (P2Y1, P2Y2, P2Y4) and thick ascending (P2Y1, P2Y2, P2Y6, P2Y7) limbs (Bailey et al. 2000, 2001a). However, functional evidence is inconsistent: basolateral application of agonists in the rat elicits intracellular Ca2+ 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 P2Y1, P2Y2, P2Y6, P2X5 and P2X7 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 variably to the basolateral or apical membrane can elicit intracellular Ca2+ 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 22Na that had been injected into the late distal tube (Bailey et al. 2001b), whereas in rats on a low-Na+ diet, in which baseline ENaC-mediated Na+ reabsorption was enhanced, ATPγS caused a significant increase in 22Na 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.


Work in the authors’ laboratory was supported by St Peter’s Trust for Kidney, Bladder & Prostate Research.

Role of endothelium in blood pressure regulation

V. Cachofeiro

University Complutense, Madrid, Spain

Neural control of renal tubular function

E.J. Johns

University College Cork, Birmingham, UK

Interactions between different mechanisms in the regulation of renal function

F.J. Salazar

Murcia, Spain

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

P.B. Persson, A. Skalweit, B. Nafz, A. Huth and B.-J. Thiele

Institute of Physiology, Humboldt University Berlin (Charité), Germany

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 unambiguously 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.

This work was supported by the German Research Foundation.

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

B. López, F.J. Fenoy, B. Arregui, F. Valero, M. García Salom and T. Quesada

Departamento de Fisiología, Facultad de Medicina, Universidad de Murcia 30100-Murcia, Spain

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 (O2−) production. Nitric oxide (NO) is an important regulator of renal function. Because the free radical O2− reacts with NO, producing peroxynitrite and decreasing its bioavailability, in the present study the role of the interactions between O2− 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 (UNaV, by −29 and −21%). ANG II also reduced renal cortical NO activity (measured electrochemically) by −9 and −23%, and lowered the urinary excretion of nitrates/nitrites (UNox) 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 UNox (+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 UNox (+26%). Pretreatment with apocynin or tempol abolished the effects of ANG II on renal cortical NO concentration, GFR, UV and UNaV. ANG II (10 nm) increased O2− 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 O2− generation by −37, −60 and −30%, respectively. Also, valsartan, apocynin and tempol abolished the effects of ANG II on O2− generation. In addition, ANG II (10−5 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 O2− that reduces renal NO bioavailability.