

Purinergic Signaling Along the Renal Tubule: The Current State of Play

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ATP-sensitive P2 receptors have been described in all mammalian nephron segments. A reductionist approach has demonstrated purinergic control of renal transporters in cell lines and heterologous expression systems. This brief update focuses on what is known of these receptors in native nephron segments (excluding the glomerulus) and outlines recent advances and possible future directions.

Although it has been known for more than half a century that extracellular purines, and specifically ATP, can affect cardiac function, it was not until 1972 that a widespread role for purinergic signaling was first proposed (17). At the time, this proposal was at odds with conventional thinking, since it was difficult to conceive of a such a ubiquitous and intracellular compound as ATP acting outside cells in a controlled and selective way; indeed, it was long held that the effects of extracellular ATP were those of its final breakdown product, adenosine. Nevertheless, 30 years on, the important actions of extracellular ATP are now widely accepted and the presence of specific cell surface receptors (P2 receptors, to distinguish them from the adenosine-selective P1 receptors; a related class of receptors is emerging that is sensitive to diadenosine polyphosphates, but it has not yet been delineated within the kidney) is clearly established (17).

P2 receptors are structurally and functionally subdivided into ionotropic P2X receptors (P2X₁₋₇) and metabotropic, G protein-coupled P2Y receptors (P2Y_{1,2,4,6,11-13}) (17). Northern blot analysis, RT-PCR, and in situ hybridization techniques have been used to map expression of specific receptor subtypes within a given tissue. More recently, antibodies (mostly polyclonal) have improved localization of P2X (and some P2Y) receptors at the protein level. However, the functional consequences of specific P2 receptor activation have been limited by the need to define different P2 receptors pharmacologically, despite the relatively poor selectivity of both agonists and antagonists for most P2 receptor subtypes, and have been complicated by interspecies differences in receptor drug sensitivities as well as the problem of ATP instability and the intrinsic activity of its breakdown products (ADP, AMP, and adenosine).

P2 receptors in the kidney

Since the renal tubule is a polarized epithelium, there may be distinct P2 receptor subtypes in each membrane domain, apical and basolateral, which can influence local transport rates of solutes and fluids. Although there are reports dating back over 20 years documenting the effects of extracellular ATP on renal tissue, the field is still at a largely descriptive and

cataloging stage. Much of our current knowledge comes from the use of kidney-derived cell lines in which P2 receptors have been identified and characterized. However, this article will focus on the function of P2 receptors in intact, postglomerular nephron segments. Studies from renal cell lines are also referred to when they provide more information than is currently available from native tissue. Due to limited space, only the most recent studies are cited, and the interested reader is referred to topical reviews for a more extensive bibliography (3, 10, 20). Figure 1 is a composite summary of the distribution pattern of P2 receptors along the mammalian nephron; for completeness, it includes the glomerulus.

The proximal convoluted tubule

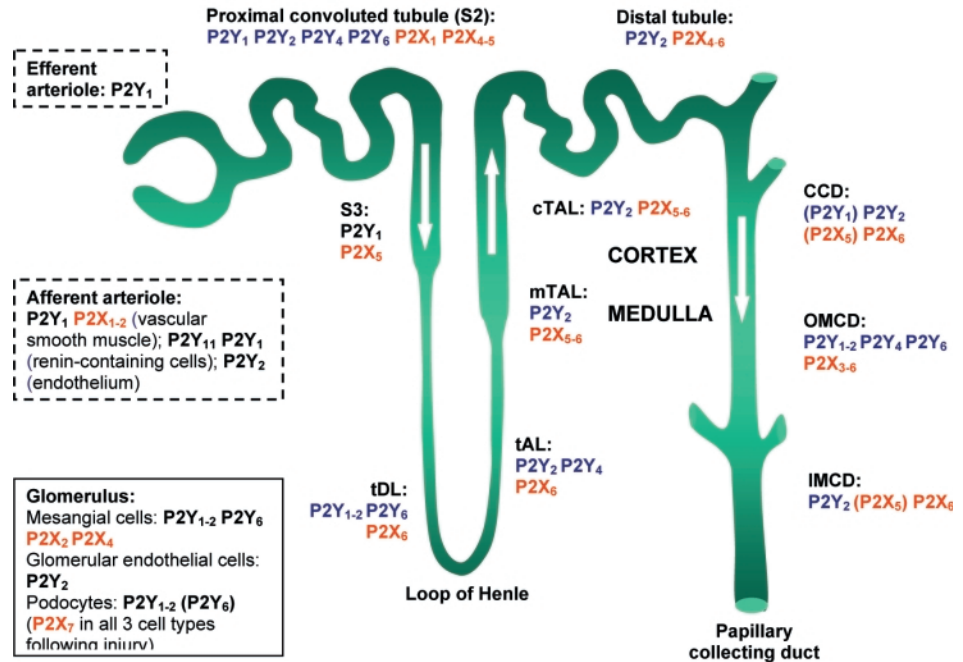
Identification. In the basolateral membrane, relative agonist potency profiles have functionally identified a P2Y₁-like receptor in the rat and *Necturus* proximal convoluted tubule (3, 4, 10, 20), and mRNA for P2Y₁ is expressed in this segment (4). The basolateral expression of P2Y₆ receptors has been suggested on a functional basis (5), and the presence of these receptors in the proximal tubule has been corroborated by detection of its mRNA (5), but not yet its protein, because a reliable antibody is not widely available. UTP and ATP_γS evoke significant calcium transients in the proximal tubule, which is in keeping with mRNA expression for both P2Y₂ and P2Y₄ receptors (4).

At the time of writing, there have only been published reports of P2X receptors (P2X₁ and P2X₄) detected in established renal cell lines, although in a preliminary immunohistochemical study of the rat nephron (10) we detected P2X₅ and P2X₆. Schweibert and colleagues (20) have undertaken the major task of establishing the quantitative distribution of mRNA for all seven P2X subtypes in primary cell cultures derived from specific regions of the nephron. Though detailed results are not yet published, it appears that proximal tubule cells express P2X₄ and P2X₅ mRNA in abundance.

Functional consequences. Basolateral chloride channels are activated by ATP in *Necturus* proximal tubule (20), which might indicate a modulatory role for extracellular ATP in vectorial transport by altering the electrical driving force across

P2 RECEPTOR SIGNALING ALONG THE NEPHRON

FIGURE 1. The distribution of P2 receptors along the nephron: an amalgam of available functional, mRNA, and protein (immuno-) detection studies showing how widespread and overlapping it is. To date, there has been no full report of their distribution in native renal tissue of any species. P2Y is shown in black and P2X in red; presence of those in parentheses is still uncertain. TAL, thick ascending limb; mTAL, medullary TAL; cTAL, cortical TAL; tAL, thin ascending limb; tDL, thin descending limb. CCD, cortical collecting duct; OMCD, outer medullary collecting duct; IMCD, inner medullary collecting duct.



the basolateral membrane. It is certainly true that ATP-induced calcium transients are more pronounced in this segment than in any other, comparable, in fact, with those induced by known regulators of proximal cell function, such as norepinephrine (4, 20). However, studies in cell lines notwithstanding, convincing evidence of a regulatory role for extracellular nucleotides in proximal tubule transport processes is still lacking. It is most likely that extracellular ATP exerts autocrine or paracrine control of renal epithelial cell function, since tightly regulated physiological release of nucleotides has been shown to occur in renal epithelial and other cell types. It is probable that ATP is released across both apical and basolateral membranes and that its local concentration in the proximal tubule could reach the micromolar level (20). In this context, researchers are beginning to uncover a subtler role for P2 receptor activation, such as modulation of hormonal action and effects on cell metabolism. For example, *de novo* renal gluconeogenesis, which can equal that occurring in the liver, is stimulated by P2Y₁ and P2Y₄ activation (15).

The loop of Henle

Identification. The anatomic loop of Henle consists of the straight portion of the proximal tubule, the thin descending and ascending limbs, and the thick ascending limb of Henle (TAL). Application of nucleotides to the basolateral membrane of the rat proximal straight tubule induced calcium transients in one study but not in another (4, 10, 20). We have detected mRNA for P2Y₆ receptors in this segment (5), but we cannot exclude the possibility that additional subtypes are also expressed. In the thin limbs of Henle, basolateral ATP and UTP were equipotent in terms of the rise in intracellular calcium concentration they induced, suggesting a P2Y₂- or P2Y₄-like receptor (4). Molecular identification of mRNAs led to the

conclusion that the responses in the descending thin limb were mediated by P2Y₂ alone, whereas both subtypes were expressed in the ascending thin limb (4). P2Y₁ and P2Y₆ receptor mRNAs have also been identified in the descending thin limb, but since known agonists for these receptors did not increase intracellular calcium concentration when applied to the basolateral membrane, we concluded (in light of the ubiquitous coupling of these receptors to phospholipase C) that they are probably located in the apical membrane only. In the rat TAL, mRNAs for four P2Y receptor subtypes have been identified (4, 5), and autoradiographic studies (3) have found dense binding sites for [³⁵S]ATPγS in this segment, suggesting expression of a P2Y₂-like receptor protein. However, it is difficult to reconcile these findings with the observation that the cortical and medullary TAL of the rat are poorly responsive (in terms of calcium transients) to basolateral application of ATP (4). In contrast, basolateral ATP and UTP consistently evoked large calcium transients in mouse TAL (3), suggesting important species differences in terms of sensitivity and/or signal transduction of P2Y receptors.

Functional consequences. It is disappointing that no study has addressed the functional consequences of P2 receptor activation in this segment of the nephron. Nevertheless, there is considerable interest in the possible role of ATP and P2 receptors as modulators, or even mediators, of tubuloglomerular feedback (TGF). TGF is the negative feedback mechanism in which the cells of the macula densa, which then signal appropriate changes in afferent arteriolar tone, sense delivery of NaCl out of the loop of Henle and thereby regulate glomerular filtration rate. In this context, we have immunolocalized P2X₁ receptors to the afferent arteriole of the rat kidney (3), and the P2X agonist β,γ-methylene-ATP causes biphasic vasoconstriction of this vessel (14). Direct assessment of TGF is obtained by measuring what is known as

the “stop-flow pressure” in the early proximal tubule, or single-nephron glomerular filtration rate (SNGFR), in response to changes in the rate of perfusion of the loop of Henle *in vivo*. Increasing the perfusion rate evokes a reduction in the proximal stop-flow pressure (or SNGFR), consistent with vasoconstriction of the afferent arteriole. That this response is markedly attenuated by simultaneous perfusion of the peritubular capillaries with either ATP or β,γ -methylene-ATP (14) implicates P2X₁ receptors in the TGF mechanism. Further support for this hypothesis has come from experiments correlating the concentration of ATP in the cortical interstitium with either inhibition or activation of TGF (16). Recent studies (6) provide evidence advocating the scheme presented in Fig. 2, whereby P2 receptors are key modulators of TGF. Increasing the concentration of NaCl presented to the luminal membrane of an isolated macula densa cell activates a large-conductance ion channel in the basolateral membrane through which ATP may pass (6). An elegant bioassay system, in which PC-12 cells expressing P2X receptors were placed in close proximity to the macula densa, unequivocally demonstrated that increased “luminal” salt delivery stimulates ATP release in quantities sufficient to produce a local concentration of ~10 $\mu\text{mol/l}$ (6).

Although the mechanism by which delivery of salt is transduced to controlled release of ATP is unknown, both mesangial cells and afferent arterioles contract in response to low concentrations of ATP. It is also worth noting that macula densa cells express P2Y receptors only in the basolateral membrane (11), perhaps indicating autocrine control of ATP release. However, it must be recognized that a wealth of scientific evidence supports adenosine, rather than ATP, as the most likely mediator of the TGF response. Pharmacological blockade of the adenosine A₁ receptor inhibits, and A₁ receptor knockout mice lack, the TGF response (19), although there is undoubtedly synergism with other vasoconstrictors, such as angiotensin II. However, it is clear that P2 receptors can modulate renal hemodynamics, and the demonstration of release of physiologically appropriate levels of ATP to act directly on P2 receptors or to provide a source of adenosine is an important finding.

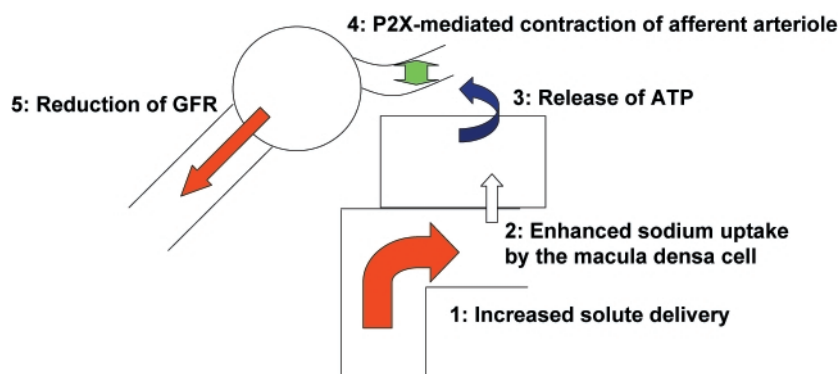
The distal tubule

Identification. The distal tubule, defined as the region of the

nephron between the macula densa and the first confluence with another nephron, consists of the distal convoluted tubule (DCT), the short connecting segment, and the initial part of the collecting duct. Early experiments suggested that the rat DCT was unresponsive to basolateral application of ATP, although more recent studies indicate the presence of an undefined P2 receptor in this membrane (4). P2Y receptors have been identified in primary cultures of rabbit DCT and in freshly isolated cells from rabbit connecting tubule (10, 20).

Functional consequences. Functional aspects of P2 receptor activation in the distal tubule have so far been limited to studies in isolated cell lines. In rabbit connecting tubule cells, ATP, when applied apically or basolaterally, inhibits both sodium and calcium reabsorption (10, 20) through diacylglycerol-mediated activation of protein kinase C. In A6 cells, an amphibian cell line that can form a polarized and high-resistance epithelium, activation of an apical P2Y₂-like receptor leads to a fourfold stimulation of chloride secretion (10), which is independent of changes in intracellular free calcium concentration. Another group reported similar findings and, in addition, suggested P2Y-mediated activation of a CFTR-like channel (1). ATP not only increased chloride secretion, but it also increased the capacitance of the apical membrane, suggesting insertion of channel-containing vesicles into the membrane in response to extracellular purines. Apical P2Y₂ receptors also promote chloride secretion in DC1 cells, an immortalized rabbit distal tubule cell line (18), although this seems to involve calcium-activated chloride channels rather than CFTR. These authors proposed that by increasing apical chloride secretion, and thereby generating an inward gradient for chloride, the apically collocated chloride/bicarbonate exchanger, and therefore bicarbonate secretion, would be activated. However, direct evidence in support of this proposal is lacking. Even so, P2 receptors could prove to be important regulators of renal acid-base balance: in transfected A6 cells, P2Y₁ receptor agonists inhibit sodium-hydrogen exchange by a cAMP/protein kinase A mechanism (2). Finally, P2X receptors have been shown to inhibit both basal and hormone-stimulated magnesium uptake in an immortalized mouse DCT cell line (7). The DCT, which reabsorbs ~15% of the filtered load, is the final site of magnesium reabsorption along the nephron, and thus ATP would influence the urinary excretion of this cation.

FIGURE 2. ATP as a possible mediator/modulator of tubuloglomerular feedback (TGF). Increased delivery of NaCl to the early distal tubule (1) increases sodium uptake across the apical membrane macula densa cells (2). Local release of ATP is stimulated (3), causing P2X-mediated contraction of the afferent arteriole (4), thereby causing a reduction in glomerular filtration rate (GFR) (5). Potential sources of ATP affecting nephron function include filtered ATP, ATP released from nerve endings (with norepinephrine), and, as already referred to, ATP released from tubular cells.



The collecting duct

Identification. Several groups have concentrated on characterizing and defining the role of P2 receptors in the collecting duct, and as a result the modulatory role of extracellular purines in this nephron segment is much better defined (see Fig. 3). P2Y₁ and P2Y₂ receptors have been identified at the molecular level (4, 10, 20), and functionally the basolateral membrane of the cortical collecting duct (CCD) and outer medullary collecting duct (OMCD) expresses both P2Y₁ and P2Y₂-like receptors (4, 10, 20). The rat inner medullary collecting duct (IMCD) also expresses a receptor with P2Y₂-like characteristics, and immunocytochemical studies have localized P2Y₂ protein to the basolateral membrane of IMCD cells (20). P2Y₄ and P2Y₆ mRNAs are also expressed in the OMCD (4, 5); basolateral application of UTP induces calcium transients in this segment, which is consistent with the expression of P2Y₄ in this membrane; however, similar studies using UDP to more selectively activate P2Y₆ indicate that this receptor subtype is not expressed at this site. Multiple P2X receptors have been identified in cell models of the CCD and IMCD (20).

P2 receptors have been identified in the apical membrane of the rat, rabbit, and mouse collecting ducts. Immunological studies localize P2Y₂ receptors to the luminal aspect of the rat principal cell (20). Imaging experiments, in which the isolated CCD was perfused *in vitro* and the calcium response to lumi-

nal purines was measured, have been used to confirm the functional presence of P2Y₂-like receptors in both intercalated and principal cells of the rat and rabbit CCD (10).

Functional consequences. Several groups have described an inhibitory effect of basolateral P2 receptors on arginine vasopressin (AVP)-stimulated osmotic water permeability in segments of the CCD and IMCD (20). Inhibition of water transport in the inner medulla occurs at low concentrations of ATP ($EC_{50} = \sim 1 \mu\text{mol/l}$) and is not seen during selective activation of apical membrane P2 receptors in the absence of AVP (8). Inhibition probably occurs at the V₂ receptor level, since ATP was shown to reduce AVP-stimulated generation of cAMP independently of any change in phosphodiesterase activity; moreover, forskolin-induced cAMP production and the effects of 8-bromo-cAMP were not impaired by P2 receptor activation. More recent experiments (10) have shown that inhibitors of protein kinase C can inhibit the effect of basolateral ATP. Inhibition of water transport by purines confirms P2 receptor localization to the principal cell of the collecting duct but does not exclude its expression by intercalated cells. Note that in the rabbit, agonists of both P2Y₁ and P2Y₂/P2Y₄ were inhibitory, whereas in the rat P2Y₁ agonists had no effect. Since P2Y₁ receptors are clearly expressed in the basolateral membrane of the rat CCD and OMCD, this suggests either a species difference with respect to the consequences of P2Y₁ receptor activation in the principal cell or that expression of P2Y₁ is limited to intercalated cells in the rat.

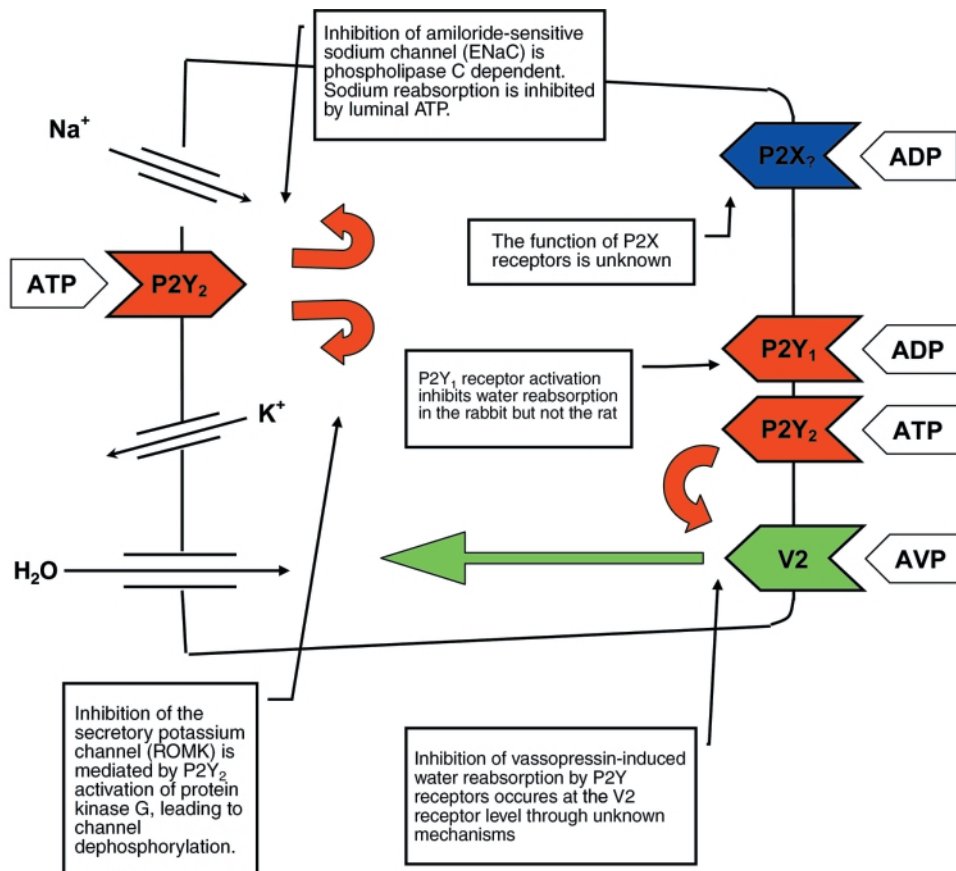


FIGURE 3. Functional consequences of P2 receptor activation in the collecting duct principal cell.

In the mouse CCD, split open to allow patch-clamp analysis of the apical membrane, ATP and UTP inhibited the small-conductance potassium channel of the principal cell (12), which accounts for much of the potassium secretion in the distal nephron. On the basis of selective inhibition of downstream signaling, the authors concluded that P2Y₂ activation increased channel dephosphorylation by enhancing protein kinase G-sensitive phosphatase activity. In the collecting duct, activation of putative luminal P2Y₂ receptors inhibits amiloride-sensitive short-circuit current (9). We have found direct evidence for an effect on transport by using an in vivo microperfusion approach in which collecting duct sodium reabsorption was assessed by recovery of microinjected ²²Na. Although P2 receptor activation was without any detectable effect in control animals, ATP_γS (a nonhydrolyzable analog of ATP) significantly increased the urinary recovery of ²²Na in rats maintained on a low-sodium diet (Shirley et al. *J Am Soc Nephrol* 12: 577A, 2001). Of some relevance is that local release of ATP and subsequent inhibition of ENaC (13) have been shown in A6 cells, supporting the notion that ATP may regulate sodium reabsorption in an autocrine/paracrine manner, possibly by flow-mediated release in vivo. So far, the functional roles of P2X receptors have not been investigated in native tissue, but a study using mIMCD-K2 cells reported inhibition of sodium reabsorption and stimulation of chloride secretion on activation of apical and/or basolateral P2X and P2Y receptors (10, 20).

Summary and future perspectives

It is clear from this review that receptors for purines and pyrimidines are strongly represented in kidney tubule epithelium, implying that there are important signaling roles for these compounds, although their multiplicity and complexity makes analysis difficult. Functionally at least, the dominant receptor types in most regions of the kidney seem to be members of the P2Y subfamily. A striking feature is the shift from P2Y₁ (ADP selective) and P2Y₆ (UDP selective) receptors in the rat proximal nephron to predominant expression of P2Y₂ receptors (UTP/ATP selective) in the loop of Henle and beyond. It is notable that P2Y receptor subtypes are often differentially expressed in apical or basolateral membranes of single epithelial cells. In most regions P2X receptors are also present, although as nonselective cation channels; defining their function will prove more difficult, and their potential role is uncertain.

Clearly, for future understanding of purine signaling in the kidney, and in particular for exploration of the therapeutic potential of purine- and pyrimidine-related compounds, the development of selective receptor agonists and antagonists that work in vivo, and without rapid breakdown, is essential. Progress is also likely if the modulation of extracellular ATP, either through potent ATPase inhibitors or by control of cellular release/uptake mechanisms, can be achieved. There is still much to be learned about this multifaceted system in the kidney, but now that we have a better appreciation of the nature and distribution of P2 receptors, a clearer understanding of their function should follow soon.

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