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P2 receptors in the regulation of renal transport mechanisms

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Vallon V. P2 receptors in the regulation of renal transport mechanisms. *Am J Physiol Renal Physiol* 294: F10–F27, 2008. First published October 31, 2007; doi:10.1152/ajprenal.00432.2007.—Extracellular nucleotides (e.g., ATP) regulate physiological and pathophysiological processes through activation of nucleotide P2 receptors in the plasma membrane. Examples include such diverse processes as communication from taste buds to gustatory nerves, platelet aggregation, nociception, or neutrophil chemotaxis. Over approximately the last 15 years, evidence has also accumulated that cells in renal epithelia release nucleotides in response to physiological stimuli and that these nucleotides act in a paracrine and autocrine way to activate P2 receptors and play a significant role in the regulation of transport mechanisms and cell volume regulation. This review discusses potential stimuli and mechanisms involved in nucleotide release in renal epithelia and summarizes the available data on the expression and function of nucleotide P2 receptors along the native mammalian tubular and collecting duct system. Using established agonist profiles for P2 receptor subtypes, significant insights have been gained particularly into a potential role for $P2Y_2$-like receptors in the regulation of transport mechanisms in the collecting duct. Due to the lack of receptor subtype-specific antagonists, however, the in vivo relevance of P2 receptor subtypes is unclear. Studies in gene knockout mice provided first insights including an antihypertensive activity of $P2Y_2$ receptors that is linked to an inhibitory influence on renal Na^+ and water reabsorption. We are only beginning to unravel the important roles of extracellular nucleotides and P2 receptors in the regulation of the diverse transport mechanisms of the kidney.

kidney; ATP; UTP; vasopressin; aquaporin; collecting duct; volume regulation

THE KIDNEYS SERVE TO MAINTAIN fluid and electrolyte homeostasis by adapting renal excretion to bodily needs. Beside systemic neurohumoral control, primary local mechanisms are important for renal function and integrity. Various autacoids are potential candidates to contribute to the signaling cascades involved in those local mechanisms. Adenosine is one such candidate, whose extracellular formation and activity depend to a significant extent on the cellular release and local breakdown of the nucleotide ATP (162). More and more evidence is now accumulating, however, that extracellular ATP and other nucleotides by themselves act in a paracrine and autocrine way to regulate a broad range of physiological and pathophysiological processes in the kidney and that they do so through the direct activation of cell membrane receptors, namely, the ionotropic P2X receptors and the metabotropic G protein-coupled P2Y receptors.

Vascular function is an important physiological process that is regulated by nucleotide receptors, and a variety of P2X and P2Y receptors are expressed in the vasculature and the glomerulus of the kidney. The current knowledge on the role of ATP and nucleotide receptors in the regulation of renal hemodynamics has recently been reviewed (45, 74). Over the last years, evidence is accumulating that the renal tubular and collecting duct system also releases nucleotides in response to physiological stimuli and that these nucleotides act locally to affect the regulation of cell volume and transport mechanisms in the kidney. Important insights have been provided by experiments demonstrating the functional expression of these receptors. This approach has depended largely on the almost ubiquitous coupling of P2Y receptors to phospholipase C and consequent mobilization of intracellular Ca^{2+} (1). The involvement of specific subtypes of P2Y receptors in the observed responses has been proposed based on their preferred responsiveness to adenine and/or uracil nucleotides or sugar nucleotides (see Table 1). Using this approach, studies in several renal cell lines as well as in primary cell culture of almost all nephron segments have identified and characterized subtypes of P2Y receptors. Especially studies by Insel and coworkers (37, 61, 113, 120, 121, 158, 188) in Madin-Darby canine kidney cells, which have a distal tubular phenotype, have implicated a role for ATP and nucleotide receptors in the regulation of epithelial cell signaling and transport mechanisms and have led the way to further studies in intact nephron segments. For detailed coverage of the studies performed in renal cell lines and cell cultures, the interested reader is referred to recent reviews (62, 141, 160).

This review will focus on studies performed in native tissue and summarizes the data on the expression and function of nucleotide receptors along the tubular and collecting duct

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Table 1. *Characteristics of cloned P2 receptors*

Receptor	Preferred Agonists	Transduction Mechanism
$P2X_1$	ATP	Intrinsic cation channel (Ca^{2+} and Na^+)
$P2X_2$	ATP	Intrinsic ion channel (particularly Ca^{2+})
$P2X_3$	ATP	Intrinsic cation channel
$P2X_4$	ATP	Intrinsic ion channel (especially Ca^{2+})
$P2X_5$	ATP	Intrinsic ion channel
$P2X_6$	Functions poorly as homomultimer	Intrinsic ion channel
$P2X_7$	ATP	Intrinsic cation channel; large pore
$P2Y_1$	ADP>ATP	G_q/G_{11}; PLC-β ↑
$P2Y_2$	ATP = UTP	G_q/G_{11} (G_i/G_o?); PLC-β ↑
$P2Y_4$	UTP (human); UTP = ATP (rodent)	G_q/G_{11} (G_i?); PLC-β ↑
$P2Y_6$	UDP>UTP>ADP	G_q/G_{11}; PLC-β ↑
$P2Y_{11}$	ATP and ADP [and UTP (172; human)]	G_q/G_{11} & G_S; PLC-β ↑
$P2Y_{12}$	ADP>ATP	G_i/others
$P2Y_{13}$	ADP>ATP	G_i/G_o/others
$P2Y_{14}$	UDP-glucose and UDP-galactose	G_i/G_o/others

Main agonist is underlined. For more details on agonists and antagonists, see Refs. 1 and 19. Adapted from Refs. 1 and 19.

system. Studies in kidney-derived cell lines are referred to when they provide critical insights to complement the data obtained in native tissue. Although the accumulating evidence implicates P2 receptors in the regulation of renal epithelial transport, because of the lack of receptor subtype-specific antagonists, the in vivo relevance of such receptors is unclear. Studies in gene-targeted mice lacking specific P2 receptors have provided first insights in this regard, and the available information is presented. However, before a discussion of these specific effects along the nephron, a brief general introduction is given on P2 receptors and the potential stimuli and mechanisms involved in nucleotide release in renal epithelia. Nucleotide release is still poorly understood but is expected to provide clues as to the functional role of the system. The interested reader is also referred to excellent recent reviews on the role of P2 receptors in epithelial transport in general (16, 87, 143) as well as in renal epithelia (140, 141, 160).

P2X and P2Y Receptors

Extracellular nucleotides can activate two families of receptors, namely, the ionotropic P2X receptors with seven subtypes being currently recognized, $P2X_{1\text{-}7}$, and the metabotropic G protein-coupled P2Y receptors with eight subtypes, $P2Y_{1,2,4,6,11\text{-}14}$ (1, 18, 33). Mammalian cells in general express one or more subtypes of nucleotide receptor together with a combination of at least nine different ecto-nucleotidases, which degrade and/or interconvert extracellular nucleotides (190). The diverse effects mediated by P2 receptors include fast signaling roles in exocrine and endocrine secretion, platelet aggregation, or neurotransmission, as well as slow (trophic) signaling roles in cell proliferation, migration, or cell turnover to name a few (1, 18, 19). P2X receptors are membrane ion channels that open in response to the binding of extracellular ATP as their principal ligand (for a review see Refs. 19 and 108). All P2X receptors are permeable to small monovalent cations; some have significant Na^+, K^+, Ca^{2+}, or anion permeability and, on activation, cause cell depolarization (see Table 1). P2Y receptors show significant differences in their agonist preference, which is used experimentally to identify subtypes (see Table 1 and for more details) (1, 19). Based on their functional coupling to particular G proteins and effector proteins, P2Y receptors can broadly be subdivided into five G_q-coupled subtypes ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$) and three G_i-coupled subtypes ($P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$) (see Table 1 and Refs. 1 and 19). In general, each P2Y receptor binds to a single heterotrimeric G protein, although $P2Y_{11}$ can couple to both G_q and G_s (187). Whereas the G_q-coupled receptors activate PLC and increase cytosolic concentrations of Ca^{2+} ($[Ca^{2+}]_i$), the G_i-coupled subtypes inhibit adenylyl cyclase and lower cAMP levels (Table 1). In some epithelia, the G_q-coupled receptors can also indirectly elicit cAMP-regulated cellular functions via activation of PLA_2, and the generation and release of prostaglandin E, which then activates EP receptors (120).

Defining the functional role of extracellular nucleotides and different P2 receptors is complicated by the lack of specific antagonists for receptor subtypes. Moreover, ecto-nucleotidases interconvert and degrade nucleotides (see below), which then can become agonists for other P2Y receptor subtypes or activate adenosine receptors. The pharmacology of purinergic signaling is further complicated by the formation of receptor homomultimers or heteromultimers. In the last 10 years, the view that G protein-coupled receptors function as monomeric proteins has been challenged by numerous studies, which suggest that these receptors exist as homomeric or heteromeric dimers or even higher-structure oligomers (3, 124). In accordance, P2Y receptor subtypes can form heteromeric complexes (for a review, see Ref. 1). Notably, adenosine is not just generated from extracellular ATP or ADP, but adenosine A_1 receptors have been shown to form a heteromeric complex with $P2Y_1$ receptors. This way an adenosine A_1 receptor is generated with $P2Y_1$ receptor-like agonistic pharmacology; i.e., ATP or ADP can bind to an adenosine A_1 receptor binding pocket, inhibits adenylyl cyclase via the $G_{i/o}$ protein-linked effector system, and the effect is sensitive to adenosine A_1 receptor blockade (183, 184). Moreover, the heterooligomerization of these two purinergic receptors is further promoted by combined agonist stimulation but not by either agonist alone (185). It is likely that also other P2Y receptors form functional heterodimers, as the rat $P2Y_2$ receptor also coimmunoprecipitated with the adenosine A_1 receptor when coexpressed in HEK293 cells (184). Thus the formation of oligomers by P2Y receptors is likely to be widespread, and also P2X receptor subunits can combine to form either homomultimers or heteromultimers (108, 166). These findings suggest a biological complexity of the system, which may serve to fine-tune the regulation of physiological processes and could account for previously unexpected pharmacological diversities. To what extent the effects of ATP or other nucleotides on kidney function depend on the presence and are mediated by G protein-coupled receptors other than P2Y, e.g., the adenosine A_1 receptor, remains to be determined.

Detection of ATP in Tubular Fluid and Renal Interstitial Fluid

Cytosolic ATP concentrations in most cell types exceeds 5 mM, and cells can release a significant proportion of ATP without being compromised (44, 80). It has been estimated that depending on the amounts of ATP released, the pericellular volume of distribution and the capacity of catabolic enzymes (especially the ecto-nucleotidases), local pericellular ATP concentrations can reach the concentrations required for nucleotide receptor stimulation (~0.1–10 μM) or even higher concentrations. EC_{50} values for ATP range from 0.7 to 15 μM for cloned $P2X_1$ through $P2X_6$ receptors (17), whereas $P2X_7$ receptors require concentrations >100 μM (95). Among the P2Y receptors, $P2Y_2$ and $P2Y_{11}$ receptors are primarily activated by ATP (Table 1). An EC_{50} value for ATP of 10 μM has been reported for the human $P2Y_{11}$ receptor (172). For the $P2Y_2$ receptor, the EC_{50} values for ATP range between 0.085 and 0.23 μM in humans and 0.7 and 18 μM in mice, with similar concentrations needed for half-maximal activation of this receptor by UTP (for a review, see Ref. 15). Sources of extracellular ATP in the kidney include perivascular and peritubular nerve terminals, circulating erythrocytes, aggregating platelets, and renal endothelial and epithelial cells. With regard to the latter, Schwiebert's group (140–142, 175) has reported that renal epithelial cultures and cell lines derived from specific nephron segments release ATP into both apical and basolateral media, with apical release predominating. Based on these in vitro data, they predicted that the luminal concentrations of ATP are highest in the proximal tubule, reaching basal concentrations of ~1 μM and that the concentrations fall to nanomolar concentrations along the nephron as the consequence of lower ATP release rates in further downstream segments. In addition, the activity of ATP-degrading ecto-nucleotidases in the luminal membranes are expected to contribute to the regulation of local concentrations of ATP as well as ADP and the generation of adenosine (see below).

More recently, Vekaria et al. (165) directly measured intraluminal ATP concentrations in micropuncture experiments in the renal tubules of anesthetized rats. They showed that proximal tubules release ATP into the lumen with ATP concentrations in the proximal tubular fluid in the range of 100–300 nmol/l, whereas concentrations in the early distal tubule were ~30 nmol/l and thus significantly lower. They also provided evidence for the presence of soluble nucleotidases in proximal tubular fluid, which could have lowered the detectable ATP concentrations especially in the distal tubular collections as those needed longer collection times (165). By use of a microdialysis method, Nishiyama et al. (106) were able to detect ATP in the interstitial fluid of the rat renal cortex and reported basal concentrations of ~7 nmol/l. Without regard to potential problems with the dialysis approach for measuring interstitial fluid components as well as possible differences in the volumes of distribution and/or ATP-degrading capacities, higher ATP concentrations in tubular fluid than in interstitial fluid would be consistent with the concept that apical ATP release is predominating, but more conclusive evidence is necessary. To which extent the measured values in the bulk phase reflect the ATP concentrations at the cell surface is unknown. Previous studies demonstrated that the measurements of nucleotides in the bulk phase could underestimate the values at the cell membrane and receptor level by at least 20-fold (66).

Expression and Potential Function of Ecto-Nucleotidases Along the Nephron

Ecto-nucleotidases degrade nucleotides, thereby modulating the ligand availability at nucleotide and nucleoside receptors (191). The enzymes involved include four families: ecto-nucleoside triphosphate diphosphohydrolases (NTPDase1, NTPDase2, NTPDase3, and NTPDase8; all members of the CD39 family); ecto-nucleotide pyrophosphatase phosphodiesterases (NPP1, NPP2, NPP3); ecto-5′-nucleotidase (CD73); and alkaline phosphatase (for a review see Refs. 190 and 191). The members of the NTPDase family hydrolyze ATP to ADP to AMP with affinities in the low-micromolar range. Members of the NPP family have a broad substrate specificity and can convert ATP or ADP to AMP as well as 3′,5′-cAMP to AMP. The K_m values for ATP are between 20 and 50 μM and thus similar to those of NTPDase family members. Extracellular AMP is converted by ecto-5′-nucleotidase to adenosine, with a K_m value also in the low-micromolar range. Finally, alkaline phosphatases are nonspecific ecto-phosphomonoesterases with a broad substrate specificity and are capable of metabolizing ATP all the way to adenosine. In contrast to the other ecto-enzymes, however, K_m values for many substrates are in the low-millimolar range (for a review, see Refs. 190 and 191).

Table 2 summarizes the expression pattern of these enzymes along the nephron. The expression pattern may provide some clues as to their function, but the current knowledge does not allow for more than speculations. As discussed above, the proximal tubule releases significant amounts of ATP into the lumen. Alkaline phosphatase has been identified in the brush-border membrane of the proximal tubule in most species and may convert ATP to ADP to AMP to adenosine. In addition, the brush border of the proximal tubule expresses ecto-5′-nucleotidase, which converts AMP to adenosine. Thus the release of ATP in the proximal tubule may serve the formation of adenosine, which is known to affect proximal tubular reabsorption (for a review, see Ref. 162). However, considering K_m values of the alkaline phosphatase for many substrates in the low-millimolar range, this enzyme may be of limited importance for the breakdown of nucleotides under physiological conditions. Furthermore, assuming little or no expression of members of the NTPDase and NPP family in the pars convoluta of the proximal tubule (Table 2), luminal ATP may be rather stable and serve to activate local P2 receptors at that site (see below). In comparison, the straight part of the proximal tubule expresses NPP3 in the apical membrane and the thin ascending limb expresses NTPDase1, both of which can convert ATP all the way to AMP. NTPDase2 and NTPDase3, both expressed in the apical membrane of the thick ascending limb (TAL) (Table 2), may further lower the luminal availability of ATP, which is consistent with direct measurements in distal tubular fluid showing significantly lower concentrations compared with proximal tubular fluid (165). Notably, NTPDases have different preferences for ATP and ADP (190). Whereas NTPDase1 hydrolyzes ATP and ADP with almost equal preference, NTPDase2 preferentially hydrolyzes ATP and NTPDase3 is a functional intermediate. As a consequence, the presence of NTPDase2 (and to a lesser

Table 2. *Localization of ecto-nucleotidases along the nephron*

	Proximal Tubule	Loop of Henle	Collecting Duct		Interstitial Cells	Peritubular Capillaries
			CCD/OMCD	IMCD		
NTPDase1		tAL (69)		Low IC, PC (164); terminal part (69)		Cortical (69, 88, 164)
NTPDase2		TAL (A, B) (164)		Low IC, PC (164)		
NTPDase3		TAL (A) (164)	IC (164)	Low PC (164)		
NPP3	PST (A) (164)					
AP	(A) (9)					
Ecto-5′ NT	Early PCT>late PCT>PST (A) (29, 39, 164)		IC (A) (29, 39, 164)	IC (A) (29, 39, 164)	css Fibroblasts (39, 85)	

AP, alkaline phosphatase; A, apical; B, basolateral; CCD, cortical collecting duct; CNT, connecting tubule; css fibroblasts, cortical stellate-shaped fibroblasts; IC, intercalated cells; IMCD, inner medullary collecting duct; low, low expression; OMCD, outer medullary collecting duct; PC, principal cell; PCT, proximal convoluted tubule; PST, proximal straight tubule; tAL, thin ascending limb; TAL, thick ascending limb. Studies were performed in rats (9, 29, 39, 69, 85, 164), mice (69), or pigs (88). All studies used immunohistochemistry except for Ref. 9, which used alkaline phosphatase activity.

extent, NTPDase3) can enhance local concentrations of ADP, which could activate $P2Y_1$ receptors, whereas the presence of NTPDase1 would convert the released ATP all the way to AMP and $P2Y_1$ receptor-mediated effects are reduced (2, 144). In this regard, it is interesting that the luminal membrane of the TAL expresses NTPDase2 and NTPDase3 but not NTPDase1, which could set the stage for luminal $P2Y_1$ receptor activation. As discussed below, mRNA for the $P2Y_1$ receptor has been detected in the TAL.

In further downstream segments, i.e., in the collecting duct, the luminal expression of ecto-nucleotidases appears rather low and primarily localized to intercalated cells (Table 2). As a consequence, ATP released into the lumen by principal cells may be rather stable and able to stimulate ATP-sensitive P2 receptors and affect transport mechanisms in an autocrine and paracrine way. Supporting this idea, ATP induces prominent effects via $P2Y_2$ receptors on sodium and fluid reabsorption in the collecting duct, as discussed below.

In the cortical interstitial space, abundant expression has been detected of NTPDase1 on peritubular capillaries (Table 2), of basolateral NPP2 in the distal tubule (48), and of ecto-5′-nucleotidase on cortical stellate-shaped fibroblasts (Table 2). This indicates that locally released ATP can be converted to adenosine and thus may activate both P2 and adenosine receptors, which may affect both glomerular and tubular function. Notably, cell surfaces can also express catalytic activity for the interconversion of nucleotides. For example, ecto-nucleoside diphosphokinase can interconvert UTP and ADP to UDP and ATP, respectively (79, 83). Moreover, kinetic analyses in human astrocytoma cells revealed that the rate of extracellular transphosphorylation exceeds that of nucleotide hydrolysis by up to 20-fold (83). Since P2Y receptors differ significantly in their specificity for adenine and uridine nucleotides and di- and triphosphates, extracellular interconversion of adenine and uridine nucleotides can play a key role in defining activities in nucleotide-mediated signaling. Whether and where ecto-nucleoside diphosphokinase activity is expressed in the kidney and along the nephron, however, are not known. Obviously, we are only just beginning to understand the dynamics and the relevance of the degradation and interconversion of nucleotides in the kidney.

Potential Stimuli and Mechanisms of ATP Release Across Apical and Basolateral Membranes in Renal Epithelia

Under physiological conditions, at least three mechanisms contribute to the regulated release of nucleotides. Exocytosis of ATP-filled vesicles is important in neurons, neuroendocrine cells, and platelets. Driven by the large concentration gradient, ATP can also be released by conductive ATP transport through ATP release channels or by nonconductive, facilitated diffusion through an ATP transporter. To which extent these mechanisms contribute to ATP release in renal epithelia is still unclear (140).

Flow-induced ATP release and the primary cilium in renal epithelia. Mechanical stimulation of ATP release has been suggested to occur in virtually every experimental protocol with cultured cells (114). Leipziger's group (65) showed that increasing the luminal flow rate (by increasing the perfusion pressure) of isolated, perfused mouse TAL triggers an apical and basolateral release of ATP, which by activating $P2Y_2$ receptors triggers Ca^{2+} signaling. Woda et al. (177) found in isolated, perfused rabbit cortical collecting ducts (CCDs) that luminal suramin, a nonspecific P2 receptor antagonist, blocked the nucleotide but not the flow-induced increases in $[Ca^{2+}]_i$. Together, these findings indicate differences in flow-induced responses, potentially including ATP release, between TAL and CCD or between mice and rabbits.

The question arises as to how flow could induce ATP release in TAL and the nature of the physiological function. Almost every vertebrate cell has a specialized cell surface projection called a primary cilium (171). Current data indicate that the primary cilium on the apical membrane of renal epithelial cells can function as a mechanosensor to detect fluid flow through the lumen (123), which can affect transport mechanism (122) or may sense renal injury and trigger proliferation (169). Moreover, a defect in cilia-mediated signaling activity is a key factor that leads to cyst formation in polycystic kidney disease (PKD), which is among the most common inherited human pathologies in the kidney (182). To test for a role of cilium-driven ATP release, Hovater et al. (54) performed elegant preliminary experiments in which they examined ATP secretion and its role in cilium-driven Ca^{2+} signaling in cilium-deficient and cilium-competent CCD principal cells from *orpk* autosomal-recessive PKD mice. They found that basal release

of ATP was not different between cilium-deficient and cilium-competent cells. However, ATP secretion stimulated by mechanical perturbations as well as flow-induced Ca^{2+} signals were more robust in cilium-competent cell monolayers. Most importantly, flow-induced and cilium-dependent increases in $[Ca^{2+}]_i$ were blocked by the ATPase/ADPase scavenger apyrase. The authors hypothesized that the intact primary cilium coalesces "releasable pools" of ATP and that cilium-driven signaling involves autocrine ATP signaling (54). Whether bending of the primary cilia triggers flow-induced ATP release in intact TAL or other nephron segments remains to be established, as well as the function of a flow-induced ATP release. As discussed in later sections, luminal ATP can inhibit Na^+ reabsorption (see below), which could help to prevent excessive reabsorption during flow-induced enhanced delivery of Na^+.

Work by Schwiebert's group (140, 142, 175) implicated a role for ATP and P2 receptors in the pathophysiology of PKD. They showed that PKD cyst epithelial cells secrete ATP into the cyst lumen where P2Y and P2X receptors are expressed and that luminal ATP stimulates Cl^- secretion. Thus ATP release can lead to the growth and expansion of renal cysts by stimulating fluid secretion. In addition, P2 signaling may contribute to the epithelial proliferation associated with PKD. More recent studies by Hillman et al. (49, 50) detected the expression of $P2X_7$ receptors in collecting duct cysts of the cpk/cpk mouse as well as in human autosomal-dominant PKD. Surprisingly, activation of $P2X_7$ receptors using an in vitro model of cyst formation from cpk/cpk mice revealed a reduction in cyst formation (51). Obviously, much has to be learned about the role of extracellular nucleotide signaling in PKD and flow-dependent ATP release.

Hypotonicity-induced ATP release in renal epithelia. Extensive in vitro studies in epithelial cells by Schwiebert's group and others (13, 142, 152, 168, 175) showed that hypotonicity and increases in cell volume are potent stimuli for ATP release at the apical and basolateral cell membrane, and by altering the transport of ions (e.g., more Cl^- efflux) and fluid, the autocrine and paracrine ATP signaling are thought to contribute to epithelial cell volume regulation. In preliminary experiments, Schwiebert's group (54) also showed that the ATP secretion stimulated by hypotonic cell swelling was more robust in cilium-competent than cilium-deficient cells. Sabirov et al. (135) suggested a role for a volume-dependent, ATP-conductive large-conductance (maxi)anion channel as a pathway for swelling-induced ATP release. However, the molecular identity and physiological relevance of this channel remain to be established. McCulloch et al. (99) reported the continuous expression of connexin hemichannels Cx30 along the luminal membrane from the medullary thick ascending limb to collecting duct of the rat, with the highest level in the distal convoluted tubule. In comparison, the expression in mice and rabbits was less and limited to intercalated cells of the collecting duct, and the authors suggested a potential role in ATP release (99). Although conclusive evidence for a role of connexin hemichannels in ATP release is lacking (80), it is intriguing that it is primarily the intercalated cells in the distal nephron that also express NTPDase and ecto-5′-nucleotidase (Table 2). This colocalization could act to spatially restrict the actions of released ATP as a paracrine or autocrine mediator of cell-to-cell signaling. Thus the molecular mechanisms that serve to release ATP in response to cell swelling remain to be established. Whereas ATP-induced changes in ion and fluid transport can serve to stabilize cell volume in the short term, extracellular nucleotides and nucleosides may also act as mitogens or cofactors of growth factors to stabilize cell volume in the long term (115, 139). A role for cell volume-dependent ATP release in collecting duct cells is proposed in later sections.

ATP release by macula densa cells and its role in tubuloglomerular communication. Tubuloglomerular feedback (TGF) is a mechanism that helps to coordinate glomerular filtration rate (GFR) with tubular transport activity or capacity and contributes to the autoregulation of GFR. In this mechanism, the tubular NaCl load at the end of the TAL is sensed by specialized tubular cells, the macula densa. These cells generate a signal to affect primarily afferent arteriolar tone, such that an inverse relationship is established between this tubular NaCl load and the GFR of the same nephron. Recently, very elegant studies by Bell and coworkers (10, 75) have shown that changes in the luminal NaCl concentration trigger the basolateral release of ATP from macula densa cells and proposed a role for a maxi-anion channel in the release mechanism. The released ATP is converted to ADP and adenosine by local ecto-NTPDase1 (111) and ecto-5′-nucleotidase (21, 56, 125, 156), and adenosine via activation of adenosine A_1 receptors induces the TGF-dependent afferent arteriolar constriction and reduction in GFR (14, 148, 163). Thus a model is envisioned in which both ATP and adenosine would be considered mediators of TGF since both are released or generated, respectively, depending on the NaCl concentration at the macula densa (161).

Since extracellular adenosine can arise from the breakdown of extracellular ATP, adenosine receptor and P2 receptor signaling events are often triggered simultaneously by concurrent increases in extracellular ATP and adenosine, and an additional direct role of P2 receptors in the mediation of TGF has been proposed (60, 107, 117). New studies show not only that adenosine derives from the breakdown of ATP but also that activation of A_1 receptors can stimulate ATP release (100, 101). Moreover, functional studies implied the expression of $P2Y_2$-like receptors at the basolateral membrane of the macula densa (90), consistent with the general concept that released ATP acts in an autocrine or paracrine fashion. The functional implication and physiological relevance of these interactions in the macula densa, however, remain to be determined.

Release of other nucleotides in renal epithelia. Besides ATP and ADP, the wide distribution of the uridine nucleotide-activated $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors in renal epithelial cells (see Table 3) suggests a potential role for UTP as an important extracellular signaling molecule. However, little information is available on the extracellular concentrations of UTP in renal tissues. Nanomolar concentrations of extracellular UTP have been detected in the medium bathing a range of different cell types (81), and these basal concentrations can be substantially increased following exposure of cells to mechanical or other forms of stress (82). Notably, a ratio of 1:3–5 for extracellular UTP/ATP was observed in both resting and mechanically stimulated nonexcitable tissue, which closely reflects the relative intracellular abundance of these nucleotides. These findings indicate a common source and possibly a common mechanism for the release of both ATP and UTP (81,

Table 3. *Expression of P2 receptors along the native tubule and collecting duct system*

	Proximal Tubules	Loop of Henle	Collecting Duct
$P2X_1$			NaCl restriction: IM: CCD, OMCD, IMCD (all IC); mRNA: CD (174)
$P2X_4$	IM: low level (159)		IM: low level (159); CCD, OMCD, IMCD (all PC and IC, PC: A/B) (174); mRNA: CD (174)
$P2X_5$	IM: PST-A (159)		IM: CCD<<OMCD<IMCD principal cells (159)
$P2X_6$	IM: low level (159)		IM: low level (159); CCD, OMCD, IMCD (all PC and IC, PC: A/B) (174); mRNA: CD (NaCl restriction) (174)
$P2X_7$			IM: mouse (50)
$P2Y_1$	IM: PST-A (159); mRNA: PCT (7)	mRNA: tDL, tAL, mTAL (7)	mRNA: OMCD (7)
$P2Y_2$	mRNA: PCT (7, 68); PST (68)	IM: tAL, mTAL (intra), cTAL (intra) (159); mRNA: tDL, tAL (7, 68), mTAL (7, 68), cTAL (68)	IM: CCD (PC and IC, PC: intra) (174); OMCD (PC and IC, PC: intra-A/B) (174); MCD IC (159), IMCD (A>B) (68); IMCD (PC and IC, PC: B>A) (174); WB: IMCD (68); mRNA: CD (174); CCD (68), OMCD (7, 68), IMCD (68)
$P2Y_4$	IM: PCT (B) (159); mRNA: PCT (7)	mRNA: tAL, mTAL (7)	IM: CCD, OMCD, IMCD (PC and IC, PC: all A) (174); mRNA: OMCD (7); CD (NaCl restriction) (174)
$P2Y_6$	mRNA: PCT, PST (8)	mRNA: tDL, mTAL, cTAL (8)	IM: CCD, OMCD, IMCD (PC and IC, PC: all intra-A) (174); mRNA: CD (174); CCD, OMCD (8)

$P2X_2$, $P2X_4$, and $P2Y_{11\text{-}14}$ have not been identified unequivocally in the kidney and therefore were not included. CD, collecting duct; cTAL, cortical thick ascending limb; IM, immunostaining; (intra), intracellular; PC, principal cell; PCT, proximal convoluted tubule; mTAL, medullary thick ascending limb; tDL, thin descending limb; WB, Western blotting. All studies were performed in rat kidney unless otherwise stated.

82) (Figs. 1 and 2). In contrast to ATP and UTP, UDP-glucose appears not to be released mechanically but only constitutively, and it is metabolized slowly (84). Very little is yet known about diadenosine polyphosphates except that they are also endogenous purinergic agonists that are generated by the kidney, including tubular structures, and that they act on P2X and P2Y receptors to alter tubular and vascular function, including potential inhibitory effects on proximal tubular reabsorption (64, 146, 147, 151). However, measurements of UTP, UDP-glucose, or diadenosine polyphosphates in tubular fluid have not been reported.

P2 Receptors in the Proximal Tubule

Initial autoradiographical studies provided evidence for binding of the stable ATP analog adenosine 5′-*O*-[3-thiotriphoshate] (ATPγS) to the basolateral membrane of the rat proximal tubule, which was competitively inhibited by UTP, suggesting the presence of $P2Y_2$-like receptors (24), i.e., $P2Y_2$ and/or $P2Y_4$, which in rodents both bind and respond to ATP and UTP at similar concentrations (Table 1). Subsequent studies demonstrated the presence of mRNA for $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors in rat proximal tubule (7, 8, 68) (see Table 3). Protein expression for $P2X_4$ and $P2X_6$ was detected by immunostaining at low levels throughout the nephron, including the proximal tubules (159). Immunostaining experiments further suggested a basolateral expression of $P2Y_4$ in the proximal convoluted tubule, whereas both $P2X_5$ and $P2Y_1$ were detected in the apical membrane of the proximal straight tubule (159).

Functional detection of P2 receptors in proximal tubule. In 1996, Yamada et al. (180) showed in isolated rabbit proximal tubules that basolateral ATP (maximum effect at 10 μM) and ADP were equally effective to induce transient increases in $[Ca^{2+}]_i$. Cha et al. (23) showed in the S1 segment of isolated rat proximal tubules that the addition of ATP, ADP, or UTP to the bath increased $[Ca^{2+}]_i$ dose dependently, indicating the presence of both $P2Y_1$ and $P2Y_2$-like receptors, i.e., $P2Y_2$ and/or $P2Y_4$ receptors. Bailey et al. (7) confirmed these findings in freshly isolated rat proximal convoluted tubules (see Table 4). As mentioned above and summarized in Table 3, basolateral protein expression of $P2Y_4$ receptors (159) as well as mRNA for both $P2Y_1$ and $P2Y_2$ receptors had been shown in proximal convoluted tubules of the rat (7, 68).

Bailey and colleagues (8) further reported that basolateral administration of UDP, an agonist preferred by the $P2Y_6$ receptor (see Table 1), induced dose-dependent calcium responses in the rat proximal tubule, which were associated with increased production of inositol phosphates. Notably, UDP-induced changes in $[Ca^{2+}]_i$ exhibited no plateau after the initial peak. Moreover, a single stimulation with a high concentration of UDP induced full desensitization of the UDP-sensitive calcium pathway but did not alter the responsiveness of the proximal tubule to ADP, an agonist preferred by $P2Y_1$-like receptors (see Table 1). Consistent with the provided mRNA expression data, the authors concluded that the $P2Y_6$ receptor is expressed in the basolateral membrane of the proximal tubule, where it is coexpressed with the $P2Y_1$ receptor (7, 8).

Activation of P2Y1 receptors inhibits bicarbonate reabsorption and P2Y2-like receptors stimulate gluconeogenesis in proximal tubules in vitro. Even though the ATP-induced calcium transients in the proximal tubule are comparable to those induced by know regulators of proximal cell function (such as norepinephrine) and are more pronounced in that segment than in any other segment of the tubular and collecting duct system (7, 8), little is known about the physiological relevance of P2Y receptors in the proximal tubule. Bailey (5) used a stationary microperfusion technique to investigate in vivo the effect of nucleotides on bicarbonate reabsorption in the rat proximal tubule. From the observed pharmacological profile, an inhibitory influence of apical $P2Y_1$ receptor activation in proximal tubule on bicarbonate reabsorption was concluded (5). Experiments in freshly prepared rat renal cortical tubule suspensions by Cha et al. (22) indicated that ATP can stimulate gluconeogenesis via P2Y receptor activation, a response associated with increases in $[Ca^{2+}]_i$. Subsequent studies by Mo et al. (103) in isolated rat proximal tubules revealed that ATP and to the same

Table 4. *Effects of P2 receptor activation in native renal tissue*

Receptor	Segment	Effect	Method (Site of Effect) Criterion for Receptor Subtype
$P2Y_1$, $P2Y_2$-like	PT	$[Ca^{2+}]_i \uparrow$	Isolated rabbit or rat PT (B) agonist profile (7, 23, 180)
$P2Y_1$	PT	Bicarbonate reabsorption $\downarrow$	Rat micropuncture in vivo (A) agonist profile (5)
$P2Y_2$-like	PT	$[Ca^{2+}]_i \uparrow$, gluconeogenesis $\uparrow$, Ca^{2+} and PLC-dependent	Rat renal cortical tubule suspensions or isolated rat PT (B) agonist profile (22, 103)
$P2Y_6$	PT	$[Ca^{2+}]_i \uparrow$ and IP $\uparrow$	Isolated rat PT (B) agonist profile (8)
$P2Y_2$-like	tDL, tAL	$[Ca^{2+}]_i \uparrow$	Isolated rat tDL or tAL (B) agonist profile (7)
$P2Y_2$	TAL	$[Ca^{2+}]_i \uparrow$	Isolated, perfused mouse TAL (A and B) agonist profile/$P2Y_2-/-$ mice (65; 116)
$P2Y_2$	TAL	Net NKCC2 activity $\downarrow$	Natriuretic response to loop diuretic, indirect evidence in $P2Y_2-/-$ mice (127)
$P2Y_2$-like	CCD	PLC $\uparrow$; $[Ca^{2+}]_i \uparrow$	Isolated, perfused rat or rabbit CCD, principal and intercalated cells (A and B) agonist profile (30, 133, 177)
$P2Y_2$-like	CCD	ENaC activity $\downarrow$; Ca^{2+} independent	Isolated, perfused CCD of mice on low NaCl diet (A and B) agonist profile (86)
$P2Y_2$-like	CD	ENaC activity $\downarrow$; involves PLC $\uparrow$	Preliminary whole-cell patch-clamp studies of principal cells of split-open rat CD (A) agonist profile (174)
P2 ?	CD	Apical plasma membrane PIP_2 levels $\downarrow$ and ENaC open probability $\downarrow$ with similar time courses	Preliminary studies on principal cells of isolated rat CD (A) ATP (Stockand JD, personal communication).
$P2X_4$, $P2X_{4/6}$	CD	ENaC-activity $\downarrow$ at extracellular $[Na^+]$ of 145 mM but $\uparrow$ at $[Na^+]$ of 50 mM	Preliminary whole-cell patch-clamp studies of principal cells of split-open rat CD (A) DIDS insensitivity/real-time PCR/immunohistochemistry (174)
P2 ?	ASDN	Na^+ reabsorption $\downarrow$	Micropuncture in rats on low-NaCl diet (A) ATP (145)
$P2Y_2$	ASDN	Aldosterone sensitivity $\downarrow$	Indirect evidence in $P2Y_2-/-$ mice (127)
$P2Y_2$-like	CCD	Small-conductance K^+ channel $\downarrow$ by PKG-sensitive phosphatase $\uparrow$	Patch-clamp analysis of principal cells in split-open mouse CCD (A) agonist profile (91)
$P2Y_2$	ASDN	K^+ excretion $\downarrow$ under control K^+ diet	Indirect evidence in $P2Y_2-/-$ mice (127)
$P2Y_2$-like	IMCD	PLC $\uparrow$; $[Ca^{2+}]_i \uparrow$, PKC $\uparrow$, cAMP $\downarrow$, AVP-stimulated osmotic water permeability $\downarrow$	Isolated, perfused CCD, OMCD, or IMCD of rabbit or rat (B) agonist profile (23, 31, 32, 67, 133)
$P2Y_2$-like	IMCD	COX-1-dependent PGE_2 release $\uparrow$; hydrated > dehydrated rats; endothelin-1 release $\downarrow$	Rat IMCD suspension (B) agonist profile (57, 149, 170)
$P2Y_2$	CD	AVP-stimulated cAMP formation and water reabsorption $\downarrow$	Evidence in $P2Y_2-/-$ mice (127)

ASDN, aldosterone-sensitive distal nephron; IP, inositol phosphates; PLC, phospholipase C; PKC, protein kinase C; PKG, protein kinase G; PT, proximal tubule; COX-1, cyclooxygenase 1.

extent UTP and the stable analog UTPγS stimulated gluconeogenesis, indicating a $P2Y_2$-like receptor and that these responses were decreased by agents that interfere with either phospholipase C activation or intracellular Ca^{2+} mobilization. Moreover, the UTP-induced gluconeogenesis was sensitive to the receptor antagonist suramin, and UDP was without significant effect on gluconeogenesis (103), indicating a role for a $P2Y_2$-like receptor (Table 4). Since $P2Y_2$ receptors are suramin sensitive (167) while $P2Y_4$ receptors are less sensitive to suramin (167) and UDP is a partial agonist on $P2Y_4$ receptors (11), the authors concluded that $P2Y_2$ receptors rather than $P2Y_4$ receptors were involved in the stimulation of gluconeogenesis.

Preliminary studies by Listhrop et al. (89) revealed an increased cortical protein abundance of the proximal tubule protein sodium-phosphate cotransporter NaPi-2 in mice lacking $P2Y_2$ receptors with no significant changes in the abundance of the sodium/hydrogen exchanger NHE3 and α_1-Na-K-ATPase, but no functional data were presented. Whether changes in NaPi-2 expression indirectly relate to a proposed role of $P2Y_2$ receptors in osteclast activity (112) has not been assessed. Overall, little is still known about the physiological and/or pathophysiological relevance of P2X and P2Y receptors in the proximal tubule, even though the luminal ATP concentrations have been suggested to be highest at this site, as outlined above.

P2 Receptors in Thin Limbs of Henle's Loop

Studies in rat kidney demonstrated the presence of mRNA for $P2Y_1$, $P2Y_2$, and $P2Y_6$ receptors in the thin descending limb (tDL) and for $P2Y_1$, $P2Y_2$, and $P2Y_4$ receptors in the thin ascending limb (tAL) (7, 8, 68) (see Table 3). Protein expression was detected by immunostaining in rat thin limbs for $P2X_4$ and $P2X_6$ and for $P2Y_2$ receptors in tAL, predominantly intracellular (159).

Functional detection of P2 receptors in thin limbs. Bailey et al. (7) measured changes in $[Ca^{2+}]_i$ in freshly isolated rat tDL and tAL in response to basolateral application of receptor agonists and found that for both segments ATP, UTP, and ATPγS were equipotent, whereas ADP had no significant effect, suggesting the presence of a $P2Y_2$-like receptor, i.e., the $P2Y_2$ and/or the $P2Y_4$ receptor (see Tables 1 and 4). The observed differences in receptor subtype mRNA expression between the two segments (see Table 3) led the authors to the conclusion that the responses in tDL were mediated by the $P2Y_2$ receptor alone, whereas both $P2Y_2$ and $P2Y_4$ subtypes may contribute in tAL (7). In comparison, basolateral administration to rat tDL of UDP, an agonist preferred by the $P2Y_6$ receptor, was ineffective. The authors speculated that the differences in the distributions of $P2Y_6$ receptor mRNA and UDP responses in tDL may indicate the presence of luminal receptors in that nephron segments (8). The functional relevance of P2 receptors in the thin limbs of Henle is unknown at this point.

P2 Receptors in TAL of Henle's Loop

Autoradiographical studies showed ATPγS binding sites in the basolateral membrane of the rat TAL, which was competitively inhibited by UTP, suggesting the presence of $P2Y_2$-like receptors (6, 24), i.e., $P2Y_2$ and/or $P2Y_4$ receptors. Subsequent studies demonstrated the presence of mRNA for $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors in medullary (mTAL) and/or cortical TAL (cTAL) in the rat (7, 8, 68) (see Table 3). Protein expression was detected in mTAL and cTAL limbs for $P2Y_2$ receptors (predominantly intracellular) and at low levels for $P2X_4$ and $P2X_6$ (159).

Functional detection of P2 receptors in TAL. In 1995, Paulais et al. (116) showed that ATP and UTP in the superfusate were equally effective in transiently increasing $[Ca^{2+}]_i$ in mouse cTAL (EC_{50}, 40 μM; sensitive to suramin) while ADP had little or no effect, indicating a role for a $P2Y_2$-like receptor. Jensen et al. (65), measuring changes in $[Ca^{2+}]_i$ in perfused mouse mTAL also provided functional evidence for the basolateral expression of $P2Y_2$ receptors as well as for an additional ATP-sensitive P2 receptor, which triggers a sustained calcium influx and a $[Ca^{2+}]_i$ plateau, possibly a Ca^{2+}-permeable P2X receptor (108). They confirmed the role of a basolateral $P2Y_2$ receptor by studying $P2Y_2$ receptor knockout ($P2Y_2-/-$) mice and in addition reported that luminal application of both 100 μM UTP or ATP triggered large increases in $[Ca^{2+}]_i$ of similar amplitude in wild-type (WT) mice but never in $P2Y_2-/-$ mice. Other nucleotides such as UDP or ADP in the same concentration were without effect, strongly suggesting that the $P2Y_2$ receptor is the only P2 receptor expressed on the luminal membrane of mouse mTAL (65) (see Table 4). Moreover, luminal $P2Y_2$ receptors promoted larger $[Ca^{2+}]_i$ elevations compared with the basolateral receptors, and $P2Y_2$ receptor activation increased $[Ca^{2+}]_i$ via release of Ca^{2+} from internal stores and likely also activation of store-operated Ca^{2+} entry (65).

Notably, Unwin's group (7) observed that changes in $[Ca^{2+}]_i$ in response to basolateral application of ATP in microdissected rat mTAL or cTAL are relatively small compared with the responses observed in rat proximal tubule, tDL, tAL, and OMCD. This is in contrast to the mouse TAL, which consistently shows large calcium transients (6, 65, 116), pointing to potential species differences with regard to sensitivity and/or signal transduction of P2Y receptors in this segment.

Bailey et al. (8) reported that basolateral administration of UDP to rat mTAL, an agonist preferred by the $P2Y_6$ receptor, was ineffective with regard to $[Ca^{2+}]_i$. Since the segment expresses $P2Y_6$ receptor mRNA, the authors proposed the luminal expression of $P2Y_6$ receptors in that nephron segment. The latter notion, however, is not supported by the above-described experiments in the mouse, although species differences cannot be excluded.

Evidence for flow-dependent ATP release and inhibition of Na^+-K^+-$2Cl^-$ cotransporter by $P2Y_2$ receptor activation. As mentioned before, increasing the luminal flow rate (by increasing the perfusion pressure) of isolated, perfused mouse TAL triggers an apical and basolateral release of ATP, which by activation of apical and basolateral $P2Y_2$ receptors triggers increases in $[Ca^{2+}]_i$ (65), but the functional role remained unclear. Studies by Rieg et al. (127) in $P2Y_2-/-$ mice revealed a greater furosemide-induced natriuresis and a greater expression of the Na^+-K^+-$2Cl^-$ cotransporter (NKCC2) compared with WT mice, indicating an inhibitory influence of $P2Y_2$ receptor activation on NaCl reabsorption in TAL (Table 4). Preliminary studies by Listhrop and colleagues (89) confirmed a greater expression of NKCC2 in the kidney of $P2Y_2-/-$ mice. These studies provide the first evidence for a potential functional role of $P2Y_2$ receptors in TAL, which may serve to inhibit and limit NaCl reabsorption in TAL during an increased flow rate and NaCl delivery.

P2 Receptors in the Distal Convoluted Tubule

Immunostaining revealed the expression of $P2X_4$ and $P2X_6$ receptors in the basolateral membrane of rat distal tubules, whereas no significant staining was detected for $P2X_5$ and $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors (159). Notably, changes in $[Ca^{2+}]_i$ in response to basolateral application of ATP in microdissected rat distal convoluted tubules were detectable (the receptor subtype remained undefined), but similar to rat TAL, the responses in $[Ca^{2+}]_i$ were relatively small compared with the responses in the proximal tubule, tDL, tAL, and outer medullary collecting duct (OMCD) (7). The functional relevance of P2X and P2Y receptors in the distal convoluted tubule is unclear.

P2 Receptors in Na^+ and K^+ Transport in the Aldosterone-Sensitive Distal Nephron

The aldosterone-sensitive distal nephron (ASDN) includes the late distal convolute tubule, the connecting tubule, and the collecting duct and expresses in its apical membrane the epithelial sodium channel ENaC, which is a primary target of the mineralocorticoid aldosterone and critically involved in the regulation of renal Na^+ reabsorption and K^+ excretion and genetic forms of arterial hypertension (58).

P2 receptor expression in ASDN. Autoradiographical studies have shown ATPγS binding sites in the basolateral membrane of rat collecting duct segments, which were competitively inhibited by UTP, suggesting the presence of $P2Y_2$-like receptors (24). Subsequent studies demonstrated the presence of mRNA for $P2Y_2$ and $P2Y_6$ receptors in rat CCD (8, 68) (see Table 3). Protein expression was detected by immunostaining at low levels for $P2X_4$, $P2X_5$, and $P2X_6$ (159). In preliminary studies, Wildman et al. (174) reported the protein expression of $P2X_1$, $P2X_4$, and $P2X_6$ as well as $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors in segments of the collecting duct in the rat kidney and confirmed the mRNA expression of these receptors in microdissected collecting ducts (see Table 3). The proposed localization of $P2Y_2$ receptors to both apical and basolateral membranes of the OMCD and that of $P2Y_4$ and $P2Y_6$ receptors primarily to the apical membrane in the CCD and OMCD is consistent with the expression pattern found in other epithelia (52, 137). The authors also proposed the protein expression of $P2X_2$, $P2X_5$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$ receptors in rat collecting duct but could not confirm the mRNA expression in microdissected collecting ducts or had no suitable primers available (174), and these were therefore not included in Table 3. Notably, the antibody used for $P2Y_{11}$ receptor detection was raised against an epitope of human $P2Y_{11}$ receptors since no murine $P2Y_{11}$ receptor gene has been detected (1). Further studies are required to clarify this issue. Notably, the studies could not detect mRNA or protein expression of $P2Y_1$ in the rat

collecting duct, and very intriguing was a proposed rise in the expression of $P2X_1$ receptors in intercalated cells in response to a low-NaCl diet (174).

Inhibition of ENaC by activation of $P2Y_2$-like receptors in vitro. In 1994, Rouse et al. (133) reported that ATP (100 nM and greater added to the bath) as well as UTP can activate the phospholipase C/Ca^{2+} signal transduction pathway in the isolated, perfused rabbit CCD. Deetjen et al. (30) confirmed the $[Ca^{2+}]_i$ response to basolateral ATP (EC_{50} of 34 μM) in the isolated, perfused rabbit CCD and also showed that the isolated, perfused CCD of the mouse responded to basolateral ATP and UTP (both EC_{50} of 23 μM) with increases in $[Ca^{2+}]_i$. Moreover, they also found that the luminal application of ATP or UTP (both with an EC_{50} of 10 μM) increased $[Ca^{2+}]_i$ in the mouse CCD with an initial peak and a plateau while luminal ADP had no effect, indicating the expression of luminal P2Y receptors, probably $P2Y_2$-like, in principal cells of mouse CCD (30). Woda et al. (177) presented functional evidence for apical $P2Y_2$-like receptors in the CCD of the rabbit. Together, these functional studies indicated the expression of both basolateral and luminal $P2Y_2$-like receptors in principal cells of CCD (Table 4).

With regard to a functional role of luminal $P2Y_2$-like receptors, Lehrmann et al. (86) demonstrated that application of both ATP or UTP (100 μM) to isolated, perfused CCD of mice kept on a low-salt diet reduces amiloride-sensitive short-circuit currents by 30–50% when applied to the apical aspect and by 20% when applied from the basolateral surface, indicating that both luminal or basolateral activation of $P2Y_2$-like receptors can inhibit ENaC-mediated Na^+ reabsorption in that segment. Notably, however, this inhibition appeared to occur independently of an increase in $[Ca^{2+}]_i$ (86) (Table 4). Studies by Cuffe and colleagues (28) using equivalent short-circuit-current measurements in M-1 mouse CCD cells revealed that extracellular ATP reduces amiloride-sensitive Na^+ absorption through activation of $P2Y_2$-like purinoreceptors located in the apical and basolateral membrane. Follow-up studies in M-1 cells and experiments by Koster et al. (76, 155) in cultured rabbit connecting tubule and CCD cells confirmed that the inhibitory effect of ATP on ENaC was independent of changes in $[Ca^{2+}]_i$.

Inhibition of ENaC by $P2Y_2$-like receptors involves activation of phospholipase C and hydrolysis of anionic phospholipids. Ma et al. (93) found in the A6 cell line, an amphibian cell line that can form a polarized and high-resistance epithelium, that the P2Y receptor agonist ATPγS by activation of phospholipase C reduces the open probability of apical ENaC. Importantly, with regard to the physiological relevance, Wildman et al. (174) concluded from preliminary whole-cell patch-clamp studies of principal cells of split-open CDs of the rat that activation of $P2Y_2$-like receptors, i.e., $P2Y_2$ and/or $P2Y_4$, contributes to ATP/UTP-mediated inhibition of ENaC and that this involves activation of phospholipase C (174) (Table 3). Ma et al. (94) further showed in A6 cells that the regulation of ENaC by phospholipase C-coupled receptors involves hydrolysis of the anionic phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) and that the effects of PIP_2 are independent of ENaC trafficking. In accordance, subsequent experiments by Kunzelmann et al. (77) in *Xenopus laevis* oocytes and M-1 mouse CCD cells provided further evidence for the following mechanism for the regulation of ENaC by $P2Y_2$-like receptors, which in a similar form has been proposed by Ma and Eaton (92): under resting conditions, the inner leaflet of the lipid bilayer contains a high concentration of PIP_2, the latter binding the NH_2 terminus of the β-subunit of ENaC, thereby maintaining the ENaC channel open. Stimulation of $P2Y_2$-like receptors activates phospholipase C, which hydrolyzes and lowers the concentration of PIP_2 with resultant decreases in PIP_2 binding to the NH_2 terminus of β-ENaC. The latter lowers ENaC activity by lowering the open probability. A role for the cytosolic portion of the NH_2 terminus of β-ENaC for PIP_2 regulation is consistent with previous studies by Yue et al. (186). The regulation of ENaC by phosphatidylinositides has recently been reviewed (92, 119). Notably, Stockand et al. have preliminary results in isolated rat collecting ducts showing that purinergic signaling rapidly decreases apical plasma membrane PIP_2 levels and ENaC open probability and that this occurs with similar time courses (Stockand JD, personal communication) (see Table 4 and Fig. 1).

Regulation of ENaC by P2X receptor activation in vitro. Besides a role for ATP- and UTP-sensitive metabotropic $P2Y_2$-like receptors, there is also evidence that activation of P2X receptor ion channels contributes to the regulation of ENaC activity. Studies in mouse inner medullary collecting duct (IMCD)-K2 cells have suggested the involvement of both P2Y (including $P2Y_2$-like) subtypes and P2X (including $P2X_4$) subtypes in ENaC inhibition (98). In accordance, Wildman et al. (173) found that activation of recombinant rat $P2X_2$, $P2X_4$, $P2X_{2/6}$, and $P2X_{4/6}$ receptor ion channels can inhibit ENaC currents in *X. laevis* oocytes. This inhibition was due to

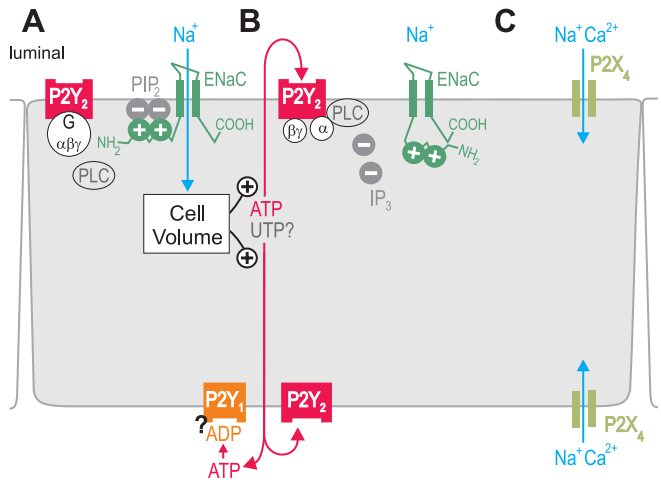

Fig. 1. Proposed model for the regulation of the epithelial sodium channel (ENaC) by $P2Y_2$ receptor activation in the aldosterone-sensitive distal nephron (ASDN). *A*: when the $P2Y_2$ receptor is not activated, the inner leaflet of the lipid bilayer contains a high concentration of negatively charged phosphatidylinositol 4,5-bisphosphate (PIP_2), which binds to positively charged regions of the NH_2 terminus of the β-subunit of ENaC, thereby maintaining the ENaC channel open. *B*: an increase in Na^+ transport triggers the basolateral and apical release of ATP (and potentially UTP?) by an as yet unknown mechanism. Stimulation of $P2Y_2$ receptors activates phospholipase C (PLC), which hydrolyzes and lowers the concentration of PIP_2. This induces a conformational change in the NH_2 terminus of β-ENaC and lowers ENaC open probability and activity (adapted from Refs. 77 and 92). *C*: $P2Y_1$ receptors have been detected in the aldosterone-sensitive distal nephron (ASDN; at least in some species), but their physiological relevance is less clear. $P2X_4$ receptors in the apical and basolateral membrane have been proposed to regulate ENaC in a Na^+- and site-dependent way. IP_3, inositol trisphosphate. See text for details.

a reduction in surface expression of ENaC and mediated by Na^+ and/or Ca^{2+} influx (173). In contrast to this "apical" inhibition, Zhang et al. (189) proposed, based on experiments in A6 cells, which are renal epithelial cells derived from *X. laevis*, that basolateral activation of $P2X_4$-like receptors stimulates ENaC activity. Moreover, they hypothesized based on the above data by Wildman et al. (173) that regulation of the site of ATP release, i.e., across the apical or basolateral membrane, could contribute to the local control of Na^+ reabsorption, with apical release inducing inhibition and basolateral release causing stimulation of ENaC. In preliminary whole-cell patch-clamp studies of principal cells of split-open CDs of the rat, Wildman et al. confirmed that apical activation of ionotropic P2X receptors, most likely $P2X_4$ and/or $P2X_{4/6}$ subtype(s), inhibits ENaC activity (with the inhibition being ~50% less than the inhibition mediated by $P2Y_2$-like receptors). To complicate things further, however, this inhibitory response was only observed at high extracellular concentrations of Na^+ (145 mM), whereas at lower concentrations (50 mM), which are more in the range of the luminal concentrations expected in the cortical aspects of the ENaC-expressing segments, apical activation of potentially the same P2X receptor subtypes potentiated ENaC activity (Table 4). In comparison, the inhibition of ENaC by activation of $P2Y_2$-like receptors was similar at both low and high extracellular Na^+ concentrations (174). Notably, $P2X_4$ receptor knockout mice are hypertensive (181), and this mouse model should be helpful in determining whether changes in ENaC regulation are involved in the hypertensive phenotype and the physiological relevance of a proposed Na^+- and site-dependent regulation of ENaC by $P2X_4$ receptor activation.

Luminal ATP inhibits Na^+ reabsorption in collecting duct, and lack of $P2Y_2$ receptors increases aldosterone sensitivity in vivo. The first in vivo evidence for an inhibitory effect of luminal ATP on Na^+ reabsorption in the collecting duct has been provided by Shirley et al. (145) in microperfusion experiments in rats maintained on a low-Na^+ diet. Subsequent experiments with more selective agonists, however, failed to identify in vivo the receptor responsible for this inhibitory effect (Table 4). More recently, we found that $P2Y_2-/-$ mice are hypertensive and have lower plasma aldosterone concentrations compared with WT mice (127). Moreover, despite lower renal expression of the α-subunit of ENaC, and thus potentially lower ENaC channel number in the apical membrane (12, 134), amiloride-sensitive Na^+ reabsorption was not significantly affected in $P2Y_2-/-$ compared with WT mice (127). These in vivo findings would be consistent with the concept that lack of $P2Y_2$ receptor activation results in greater ENaC open probability, while compensatory decreases in circulating aldosterone concentrations and ENaC expression serve to normalize net ENaC activity. Moreover, much smaller increases in plasma aldosterone were required to adapt renal Na^+ excretion to restricted intake in $P2Y_2-/-$ mice, suggesting a greater aldosterone sensitivity and facilitation of renal Na^+ retention (127) (Table 4). This again would be consistent with a greater open probability of ENaC in the absence of $P2Y_2$ receptors, since in this situation a smaller aldosterone-induced increase in ENaC number in the apical membrane would be sufficient to induce a similar increase in total ENaC activity compared with WT mice (see Fig. 1). Further experiments are required to demonstrate that the ENaC open probability is in fact increased in mice lacking $P2Y_2$ receptors.

A role for $P2Y_2$-like receptors in the inhibition of K^+ secretion in ASDN. Lu et al. (91) performed patch-clamp analysis of the apical membrane in the split-open mouse CCD and observed that ATP and UTP inhibited the small-conductance K^+ channel of principal cells, which together with the large-conductance (BK) Ca^{2+}-activated K^+ channel (128) accounts for much of the K^+ secretion in the ASDN. Additional experiments with selective inhibition of downstream signaling indicated that activation of $P2Y_2$-like receptors inhibited the small-conductance K^+ channel by enhancing protein kinase G-sensitive phosphatase activity, thereby increasing channel dephosphorylation (91) (Table 4). Whether P2Y receptor activation also inhibits (or stimulates) the BK channel remains to be determined. The latter is of particular interest since an increase in tubular flow cannot only increase ATP release and $P2Y_2$ receptor activation (65) but also stimulates K^+ secretion via the BK channel in the ASDN (118, 128, 176). Notably, an increase in intracellular Ca^{2+} associated with bending of the primary cilium is the cause of the hyperpolarization and activation of Ca^{2+}-activated K^+ channels in Madin-Darby canine kidney cells (122). The role of ATP and P2Y receptors has not been tested in this response.

Rieg et al. (127) showed that mice lacking $P2Y_2$ receptors have unaltered total renal excretion of K^+. However, basal plasma concentrations of both K^+ and aldosterone, which are primary stimulators of renal K^+ excretion, were lower in $P2Y_2-/-$ compared with WT mice (127). These findings indicate a facilitated renal K^+ excretion in $P2Y_2-/-$ mice under standard K^+ diet and are consistent with the above outlined inhibitory influence of $P2Y_2$-like receptor activation on the small conductance K^+ channel in principal cells of mouse CCD (Table 4). Another potential factor in those animals, however, is a proposed greater flow rate of early distal tubules (see below), which may enhance K^+ secretion in the ASDN. Of note, the facilitation of urinary K^+ excretion in $P2Y_2-/-$ mice dissipated in response to a high-K^+ diet. Thus, conditions requiring high rates of renal K^+ excretion blunt the inhibition of such excretion by $P2Y_2$ receptors (127).

P2 Receptors and Water Transport in the Collecting Duct

Body water balance is tightly regulated by arginine vasopressin (AVP), which acts via vasopressin V_2 receptors (V2R) to regulate water reabsorption by the collecting duct. Activation of basolateral V2R stimulates cAMP formation and activation of protein kinase A, which regulates acutely the water permeability of the collecting duct by phosphorylating and trafficking of aquaporin-2 (AQP2) from intracellular vesicles to the apical plasma membrane. cAMP may also leave the cell and be converted to adenosine (63), which by activation of adenosine A_1 receptors can inhibit adenylyl cyclase and induce a feedback inhibition of AVP-induced water permeability (178, 179) (see Fig. 2). The long-term adaptational changes in body water balance are controlled in part by regulated changes in AQP2 and AQP3 expression levels (for a review, see Ref. 105).

P2 receptor expression in the medullary collecting duct. Studies in the rat demonstrated the presence of $P2Y_2$ receptor mRNA and protein in the IMCD (68), whereas $P2Y_1$ receptor

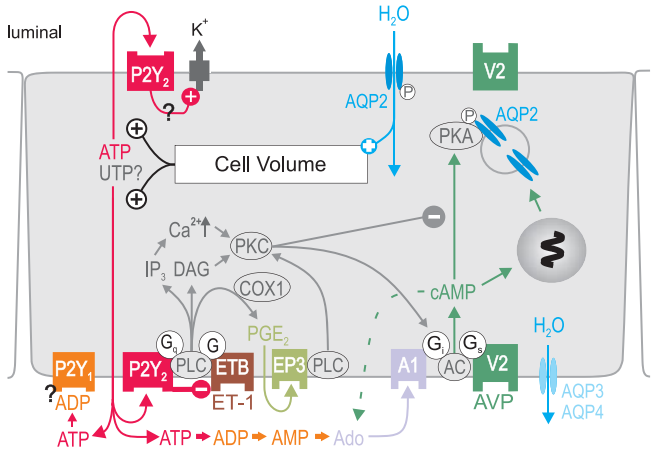

Fig. 2. Proposed model for cell volume-dependent ATP release and water transport inhibition by $P2Y_2$ receptor activation in inner medullary collecting duct (IMCD) cells. Activation of vasopressin V_2 receptors (V2) stimulates aquaporin-2 (AQP2)-mediated water entry, which increases cell volume. The latter increases basolateral and apical release of ATP (and potentially UTP?) by an as yet unknown mechanism. $P2Y_2$ receptor activation inhibits water reabsorption and stimulates volume-regulatory K^+ channels, as shown in other cell types. These effects help normalize cell volume and accelerate the excretion of free water in response to water loading before circulating arginine vasopressin (AVP) levels fall. In the latter situation, the relative hypotonic extracellular fluid increases cell volume. $P2Y_1$ receptors and several P2X receptors (not shown) have been detected in IMCD (at least in some species), but their physiological relevance is less clear. To which extent extracellular ATP is degraded by ecto-nucleotidases to ADP, AMP, and adenosine (Ado) at the luminal (not shown) and basolateral surface in the inner medulla in vivo is unclear. A1, adenosine A_1 receptor; AC, adenylyl cyclase; COX-1, cyclooxygenase 1; DAG, diacylglycerol; EP3, PGE_2 E3 receptor; ET-1, endothelin 1; ETB, endothelin B receptor; G_i, inhibitory G protein; G_q, G_s, stimulatory G protein; P, phosphorylation. See text for details.

mRNA was detected in OMCD (7). Immunostaining indicated the expression of $P2Y_2$ receptors in intercalated cells of the medullary collecting duct (159) as well as in both apical and basolateral membranes of principal cells of the IMCD (68, 174) (see Table 3). In addition, preliminary studies by Wildman et al. (174) localized both the $P2Y_4$ and $P2Y_6$ receptors to the apical membrane of IMCD; neither mRNA nor protein expression of $P2Y_1$ receptors could be detected in this segment. The proposed localization of $P2Y_2$ receptors to both apical and basolateral membranes but that of $P2Y_4$ and $P2Y_6$ receptors primarily to the apical membrane in IMCD is consistent with the expression pattern for these receptors found in other epithelia (52, 137). Besides the protein expression of $P2X_4$ and $P2X_6$ (159) [preliminary studies indicated both apical and basolateral staining (174)], IMCD expressed significant amounts of $P2X_5$ (159).

Inhibition of AVP-induced water permeability by P2Y₂-like receptor activation in vitro. In 1994, Ecelbarger et al. (31) reported that ATP (1–100 μM in superfusate) as well as UTP and ATPγS mobilize intracellular Ca^{2+} in the isolated rat terminal IMCD. Cha et al. (23) showed in isolated rat OMCD that addition of ATP, ADP, or UTP to the bath increased $[Ca^{2+}]_i$ dose dependently, indicating the presence of both $P2Y_1$ and $P2Y_2$ receptors. Rouse et al. (133) reported in 1994 that ATP (100 nM and greater added to the bath) as well as UTP can inhibit AVP-stimulated osmotic water permeability in the isolated, perfused CCD of the rabbit by activation of the phospholipase C/Ca^{2+} signal transduction pathway. Kishore et al. (67) subsequently demonstrated in the isolated, perfused terminal IMCD of the rat that both ATP and UTP (10 μM in bath) decrease the AVP-stimulated osmotic water permeability by ~40%, while ADP is ineffective. Moreover, they showed that ATP acts by decreasing cellular cAMP levels in a PKC-dependent fashion, most likely resulting from activation of the phosphoinositide signaling pathway (67). Previous work by Teitelbaum and Berl (153, 154) had shown that increased $[Ca^{2+}]_i$ inhibits AVP-stimulated adenylyl cyclase activity in rat IMCD cells by activation of PKC and that this activation requires the intact inhibitory guanine nucleotide binding protein Gi. Teitelbaum proposed, based on his own and others' data, that the inhibitory effect of PKC on AVP-stimulated water permeability also included an unidentified post-cAMP site (see below). Together, these studies indicated that activation of $P2Y_2$-like receptors can inhibit AVP-stimulated water transport in IMCD by a mechanism that involves activation of phospholipase C, an increase in $[Ca^{2+}]_i$, and activation of PKC, which may act at both pre- and post-cAMP sites (see Table 4 and Fig. 2).

Moreover, evidence was provided by Kishore et al. (70) that the expression of $P2Y_2$ receptor protein expression in the inner medulla of the rat is enhanced by hydration for 48 h (by providing sucrose water) whereas mRNA expression was unaltered. In comparison, infusion of the V2R agonist dDAVP (20 ng/h, for 5 or 6 days) decreased $P2Y_2$ receptor protein abundance by ~60% in inner medulla of rats, and the authors speculated that this may be due to a direct effect of dDAVP or a dDAVP-induced increase in medullary tonicity (150).

Local formation of PGE_2 can inhibit water, Na^+, and urea uptake in collecting ducts (104, 130, 132). Ecelbarger et al. (31) had shown that the ATP-induced mobilization of intracellular Ca^{2+} in the rat IMCD was attenuated by unselective blockade of cyclooxygenase (COX), indicating relevant interactions between ATP and prostaglandins. Welch et al. (170) reported in freshly prepared rat IMCD suspensions that ATPγS enhances the release of PGE_2 in a time- and concentration-dependent fashion, a response that was more sensitive to inhibition of COX-1 than COX-2. In subsequent studies, Sun and colleagues (149) found that hydrated rats have higher and dehydrated rats have lower urinary PGE_2 excretion compared with rats on normal-water intake. This was associated with a greater ATPγS-induced stimulation of PGE_2 release in freshly isolated IMCD preparations in hydrated rats compared with control rats, whereas the response to ATPγS was blunted in IMCD of dehydrated rats. Additional experiments indicated a role for COX-1 in ATPγS-induced PGE_2 release in hydrated rats (149). Together, these experiments have provided evidence that local ATP release and activation of $P2Y_2$-like receptors can stimulate the formation and release of PGE_2 (Table 4 and Fig. 2). The latter binds to EP receptors to activate phospholipase C, and by increasing $[Ca^{2+}]_i$ and activating PKC can inhibit AVP-induced water permeability at a post-cAMP site (104). Moreover, based on the above-described upregulation of $P2Y_2$ receptors and ATP-induced PGE_2 release in IMCD of hydrated rats, Kishore and colleagues (70) proposed that an AVP-independent regulation of IMCD function by $P2Y_2$ receptors may serve to facilitate renal free water excretion.

Studies by Edwards (32) in the isolated perfused rat IMCD showed that basolateral, but not apical, ATP inhibited the increase in water permeability in response to AVP in the bath.

This was unexpected based on the apical and basolateral expression of $P2Y_2$ receptors. AVP had not been applied to the lumen in these experiments. Since V2R are expressed in both the basolateral and luminal membrane (138), it remains to be determined whether luminal ATP requires coactivation of luminal V2R to inhibit water transport.

Inhibition of AVP-induced water transport by $P2Y_2$ receptor activation in vivo. Consistent with an inhibitory influence of $P2Y_2$ receptors on cAMP-mediated regulation of AQP2 in vivo, we found that $P2Y_2-/-$ mice have greater basal urinary cAMP excretion and renal medullary expression of AQP2 than WT mice despite similar AVP levels (127). The basal urinary excretion of fluid as well as urinary osmolality and electrolyte-free water clearance (Cl^e-H_2O) were not different between $P2Y_2-/-$ and WT mice. However, experiments with acute pharmacological inhibition of the V2R revealed a significantly greater diuresis and Cl^e-H_2O associated with lower urinary osmolality in $P2Y_2-/-$ mice compared with WT mice, whereas urinary cAMP excretion was reduced in both genotypes and was no longer different. Additional experiments in freshly isolated IMCD revealed that cAMP formation induced by the V2R agonist dDAVP was inhibited by ATPγS in WT but not in mice lacking $P2Y_2$ receptors (127). These studies provided evidence that V2R stimulation triggers $P2Y_2$ receptor-mediated inhibition of cAMP formation. We propose that this involves cell volume-regulated changes in ATP release as illustrated in Fig. 2 and discussed further below. Thus $P2Y_2$ receptor activation can feedback inhibit cAMP-dependent effects of AVP and, as a consequence, $P2Y_2-/-$ mice have greater expression of AQP2 and greater reabsorption of fluid in the collecting duct compared with WT mice (127) (Fig. 2 and Table 4). Preliminary studies by Kishore et al. (71) confirmed an increased expression of AQP2 in renal medullas of $P2Y_2-/-$ mice. The upregulation of AQP2 expression in these knockout mice may relate to the well-described chronic transcriptional activation of the AQP2 gene by cAMP via direct (cAMP-responsive element-mediated, see Fig. 2) and indirect (hypertonicity-mediated) mechanisms (55, 97). An increased water reabsorption in the collecting duct without effects on urinary flow rate or osmolality in $P2Y_2-/-$ mice indicated a greater delivery of a more hypotonic fluid to the distal tubule, which was proposed to be the consequence of the integrated renal phenotype of these mice (127) (see below).

Proposed role for cell volume-dependent ATP release in collecting duct cells. ATP detected in urine is likely to result from renal epithelia, which release ATP across the basolateral and apical membrane (see above), and perhaps from the lower urinary tract, where the release of ATP could be stimulated by distension or hypotonicity (35, 72). Recent experiments in mice by our group revealed that acute oral water loading and acute pharmacological blockade of V2R induced similar increases in urinary flow rates and decreases in urinary tonicity and thus potentially exposed the lumen of collecting ducts to similar increases in flow rate and the urothelial cells of the lower urinary tract to similar distension and hypotonicity. The observed effects on urinary ATP excretion, however, were actually opposite in response to these two maneuvers, indicating a predominant role for other mechanisms in the control of ATP secretion under these conditions (127). V2R blockade reduced urinary ATP excretion, indicating a basal activation of ATP release by V2R activation. As discussed above, cell volume-regulated ATP release is a well-established phenomenon (136). In this regard, lower urinary ATP excretion could reflect V2R blockade-induced reductions in cell volume, which is caused by blocking the apical water entry, thus off-setting the basal, AVP-, and cell volume-induced ATP release. In contrast, acute water loading enhanced urinary ATP excretion, which may reflect increases in collecting duct cell volume due to a reduction in extracellular tonicity, while the water permeability of the cell membrane is still high due to the slower fall in vasopressin activity. By inhibiting AVP-induced water permeability, the released ATP can serve to stabilize cell volume and accelerate the diuretic response to water loading. Notably, greater AQP2 expression and greater basal fluid reabsorption in collecting ducts of mice lacking $P2Y_2$ receptors were associated with greater basal urinary excretions of ATP. Moreover, urinary ATP excretion in these knockout mice was similar compared with WT mice during inhibition of water transport by V2R blockade, modestly greater under basal conditions, and much greater in the knockout mice in response to acute water loading (127). These in vivo findings would be consistent with the concept that a lack of $P2Y_2$ receptor-mediated inhibition of water transport and activation of regulatory volume decrease results in greater cell volumes, causing greater ATP release in the collecting duct (see Fig. 2). This idea is consistent with an autocrine/paracrine signaling role of ATP release and P2Y/$P2Y_2$ receptor activation, as suggested by studies of volume regulation in biliary epithelial cells (131), intestine 407 cells (110), airway epithelia (109), rat hepatoma cells (168), and human hepatocytes (34), but needs to be confirmed with more direct assessment of cell volume regulation in mice lacking $P2Y_2$ receptors.

Evidence for facilitated water excretion in response to water loading in mice lacking $P2Y_2$ receptors. The concept proposed by Kishore and colleagues (70) that activation of $P2Y_2$ receptors serves to facilitate renal free water excretion in hydrated conditions would predict that the ability to enhance free water excretion (and possibly PGE_2 release) in response to water loading is attenuated in mice lacking $P2Y_2$ receptors. On the other hand, the greater delivery of a more hypotonic fluid to the distal tubule of $P2Y_2-/-$ mice could facilitate free water excretion. To further test this issue, we performed acute oral water loading experiments in mice lacking $P2Y_2$ receptors (127). We observed that the water load-induced increase in Cl^e-H_2O was similar to that in WT mice, even though urinary vasopressin and cAMP excretion remained significantly higher in the knockout mice, and this was associated with significantly greater increases in urinary PGE_2 compared with WT mice (127). In accordance with these acute experiments, preliminary studies with chronic water loading (0.6 M glucose in drinking water for 7 days) showed that this maneuver induced a significantly greater increase in Cl^e-H_2O in $P2Y_2-/-$ mice compared with WT even though urinary excretion of vasopressin remained greater in $P2Y_2-/-$ mice. Moreover, as in response to acute water loading, urinary PGE_2 was significantly greater in $P2Y_2-/-$ mice compared with WT in response to chronic water loading (126). Thus, in the absence of $P2Y_2$ receptors, a lesser suppression of the vasopressin/cAMP axis appears sufficient to induce the same increase in free water excretion as in normal mice, indicating a facilitated rather than attenuated ability to enhance free water excretion in response to this

maneuver. A facilitated response may relate to a greater delivery of a more hypotonic fluid to the distal tubule and possibly to the greater PGE_2 release in mice lacking $P2Y_2$ receptors. The latter response was unexpected based on the concept proposed by Kishore and colleagues (70) for a role of $P2Y_2$ receptors in the release of PGE_2 in the collecting duct (see above). The greater PGE_2 release in mice lacking the $P2Y_2$ receptor implied that also other P2 receptors contribute to ATP-induced cellular PGE_2 release and/or the lack of $P2Y_2$ receptors enhanced the release of PGE_2 by other mechanisms, as discussed in the following.

The renal medullary endothelin system is an important determinant of renal Na^+ and water excretion, with activation of ETB receptors triggering natriuresis and diuresis (for a review, see Ref. 73). Acute water loading increases endothelin-1 formation in IMCD, which inhibits AVP-induced cAMP formation and accelerates the excretion of free water in the face of falling, yet still antidiuretic, plasma vasopressin levels (40). Thus activation in IMCD of both $P2Y_2$ receptors and the endothelin system can facilitate free water excretion. Indicating a potentially more complex interaction between these two systems, a recent study by Huges and colleagues (57) provided evidence that ATP acts, at least in part, through activation of $P2Y_2$-like receptors to inhibit endothelin-1 release and mRNA levels in rat IMCD (IC_{50} ~1 μM ATP) (Fig. 2), an effect that is independent of COX activity and nitric oxide metabolites (57). Moreover, ATP inhibited endothelin-1-induced increases in $[Ca^{2+}]_i$ in the isolated rat OMCD (23). Whether ATP-induced inhibition of the endothelin system is physiologically relevant and results in greater activation of the endothelin system in response to water loading in $P2Y_2-/-$ mice, which could enhance ETB-mediated PGE_2 formation and free water excretion, remains to be determined.

Studies in P2 Receptor Knockout Mice

Studies in mice lacking specific genes can provide significant insights into the qualitative and quantitative relevance of a given gene product and can help to identify potential compensation mechanisms. Knockout mice for various P2 receptors have been generated and provided exciting new insights with regard to various organ functions. Examples include the findings that activation of $P2X_2$ and $P2X_3$ receptors is crucial for communication from taste buds to gustatory nerves (36), that $P2X_7$ receptor activation is proinflammatory and enhances nociceptive sensitivity (26, 78), or that $P2Y_2$ receptors are involved in neuronal growth (4) or neutrophil chemotaxis (25). Significant insights have also been provided into the role of $P2Y_2$ receptors in nonrenal epithelia like K^+ secretion in the colon (96), Ca^{2+} signaling in lung fibroblasts and airway epithelial cells (53), and Cl^- secretion in the trachea and gallbladder (27), whereas $P2Y_4$ receptors have been implicated in Cl^- secretion in the jejunum and colon (41, 129) and K^+ secretion in the colon (96). However, little has been reported about kidney function in P2 receptor knockout models. Inscho et al. (59) showed that $P2X_1$ knockout mice have an impaired renal autoregulation, and Goncalves et al. (42) provided the first evidence that $P2X_7$ receptors are involved in renal macrophage infiltration, collagen deposition, and apoptosis in response to ureteral obstruction in mice, which may relate to a potential role of $P2X_7$ receptors in PKD (see above). As discussed, Leipziger's group (65) has used the $P2Y_2$ receptor knockout mouse to show flow-induced apical and basolateral ATP release and activation of this receptor in TAL. We have performed the first studies on kidney function in mice lacking $P2Y_2$ receptors (127), as discussed in the above segment-specific sections, and these phenotypes are integrated with regard to blood pressure regulation in the following.

The blood pressure phenotype and a proposed integrated model of renal function in mice lacking $P2Y_2$ receptors. The absence of $P2Y_2$ receptors results in salt-resistant hypertension (127). Heart rate was reduced and inversely related to salt intake in $P2Y_2-/-$ mice, indicating an efficient activation of baroreceptors in these mice in response to enhanced salt intake. A dysfunction of arterial baroreceptors has been implicated in genetic forms of salt-sensitive hypertension in rats (43, 102, 157) and humans (38), and thus hypertension in $P2Y_2-/-$ mice may be salt resistant because the baroreceptor response to variations in salt intake is intact, allowing the effectively increased renal salt excretion by lowering aldosterone and the antinatriuretic tone of the sympathetic nervous system.

As outlined above, $P2Y_2-/-$ mice have greater expression of NKCC2 and AQP2 and greater Na^+ and fluid reabsorption via these transporters, respectively. We speculate that an increased ENaC open probability also facilitates Na^+ reabsorption in these mice (see above and Fig. 1). Impaired renal Na^+ and fluid excretion increases the effective circulating volume, which increased blood pressure and suppressed renin and aldosterone concentrations and possibly renal sympathetic nerve activity in $P2Y_2-/-$ mice and Suppression of aldosterone downregulated ENaC expression, which normalized net Na^+ reabsorption via ENaC in the face of a potentially greater ENaC open probability. We speculate that both the greater blood pressure and an inhibited renal sympathetic nerve activity inhibited proximal tubular reabsorption of Na^+ and fluid in $P2Y_2-/-$ mice. Since GFR and thus the filtration of Na^+ and fluid was normal in $P2Y_2-/-$ mice, this induced greater deliveries of Na^+ and fluid out of the proximal tubule. Greater Na^+ reabsorption via NKCC2 in water-impermeable TAL normalized net Na^+ delivery but caused the delivery of greater amounts of more hypotonic fluid to the early distal convoluted tubule. When the vasopressin system and thus the distal water permeability were suppressed, the latter facilitated free water clearance in knockout mice (see above). Under basal conditions, however, the excess water was reabsorbed via the increased AQP2 activity (Fig. 2), such that net urinary Na^+ and fluid excretion was normal in $P2Y_2-/-$ mice (127). This interpretation, which is graphically illustrated in the original publication, can explain the compensated basal phenotype and the observed experimental phenotypes of these mice, which have distinct defects in renal sodium and water transport.

Impaired renal Na^+ excretion can also induce secondary increases in peripheral resistance (47). Moreover, studies in knockout mice have implicated $P2Y_2$ receptors in ATP-evoked relaxation of the murine aorta (46). However, the role of $P2Y_2$ receptors in small arteries, which are the primary determinants of peripheral resistance, remains to be assessed. Based on the phenotype in knockout mice, we proposed that a blunting in $P2Y_2$ receptor expression or activity is a new mechanism for salt-resistant arterial hypertension. Future experiments will need to address the therapeutic potential and the question of

whether genetic polymorphisms of $P2Y_2$ receptors (20) contribute to the pathophysiology of essential hypertension.

Summary and Perspectives

We are only beginning to understand the role of extracellular nucleotides and P2 receptors in the complex regulation of the heterogeneous transport mechanisms that occur along the tubular and collecting duct system. We are in the process of generating a more complete picture of the localization of P2 receptor expression in the kidney under basal condition but still know very little about how the expression is regulated and affected by physiological and pathophysiological conditions. Similar considerations apply to the luminal availability of ATP, which has been detected under basal conditions, but relatively little is known about its regulation, the stimuli for its release, or the pathways involved in the release. Even less is known about the availability and physiological function of other nucleotides like ADP, UTP, or UDP-glucose, or about the role of nucleotide degradation and interconversion in defining the activities of nucleotide and adenosine receptor-mediated signaling in the kidney. It remains to be established how cells use multiple subtypes of P2Y receptors that appear to be redundant with regard to G protein coupling and second messenger generation (e.g., $P2Y_2$ and $P2Y_4$ receptors) as well as receptor homomultimers and heteromultimers for the regulation of specific functions. The first evidence indicated specific functions of P2 receptors in the proximal tubule, like inhibition of bicarbonate transport by $P2Y_1$ receptors and stimulation of gluconeogenesis by $P2Y_2$-like receptors, or inhibition of NKCC2-mediated transport in TAL by $P2Y_2$ receptors. Much has been learned from in vitro studies about the role of $P2Y_2$-like receptors in the regulation of the epithelial sodium channel ENaC and the regulation of water transport in the collecting duct. Moreover, the first studies in knockout mice provided evidence for an in vivo role of $P2Y_2$ receptors in the regulation of renal reabsorption of Na^+ and fluid. To better characterize the functional role of P2 receptor subtypes, specific antagonists are very much needed. In the meantime and in addition, studies in gene knockout mice should prove very helpful in further delineating the role of P2 receptors in the regulation of renal transport mechanisms as well as renal hemodynamics under physiological and pathophysiological conditions and assessing the therapeutic potential of the system.

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