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P2X Receptors: Epithelial Ion Channels and Regulators of Salt and Water Transport

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Abstract
When the results from electrophysiological studies of renal epithelial cells are combined with data from in vivo tubule microperfusion experiments and immunohistochemical surveys of the nephron, the accumulated evidence suggests that ATP-gated ion channels, P2X receptors, play a specialized role in the regulation of ion and water movement across the renal tubule and are integral to electrolyte and fluid homeostasis. In this short review, we discuss the concept of P2X receptors as regulators of salt and water salvage pathways, as well as acknowledging their accepted role as ATP-gated ion channels.

Ion Channels in Polarized Renal Epithelial Cells

Polarized renal epithelial cells serve to maintain electrolyte and fluid balance in the body by regulating the movement of ions and water across the surface of the kidney tubule. Epithelial cells are involved in either the net absorption or secretion of ions, or absorption of water – a role dependent on the concerted actions of channels, co-transporters, exchangers and pumps that are selectively distributed to the apical and basolateral membranes. These primary structures are regulated, in turn, by a complex series of intracellular and extracellular signaling pathways which come under genomic and non-genomic control. Voltage-independent ion channels (e.g., ENaC, ROMK1 and CFTR), voltage-dependent ion channels (e.g., the CLC family) and cAMP-dependent water channels (i.e., the AQP protein family) exert a considerable influence on net ion and fluid transport. Their importance in solute and water salvage is apparent from a number of inheritable human diseases that disrupt the expression of normal channels in the kidney (e.g., Liddle's and Dent's diseases, cystic fibrosis, Bartter's syndrome types II and III, pseudohypoaldosteronism type I, autosomal dominant polycystic kidney disease type II, and autosomal nephrogenic diabetes insipidus).

Voltage-independent and voltage-dependent ion channels are not the only types of ion channels found in epithelial cells. Additionally, a number of ligand-gated ion channels (LGICs) have been identified and they are activated by substances more commonly associated with neurotransmission (e.g., NMDA, $GABA_A$ and nACh receptors). This list of LGICs now includes the surface receptors known as P2X receptors, which are activated principally by extracellular adenosine 5′-triphosphate (ATP). Experimental observations have led us to propose that P2X receptors regulate the opening and trafficking

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of ion and water channels in the kidney tubule. Evidence for this advanced role is also supported by findings from in vivo microperfusion of the kidney tubule with ATP and its analogues, which have been shown to alter the degree of electrolyte and water salvage in salt-depleted animals. In this short review, we discuss a novel role of P2X receptors as regulators of epithelial cell transport, in addition to their widely accepted role as membrane pathways for Ca^{2+} and Na^{+} influx.

Introduction to P2X Receptors

P2X receptors represent the LGIC subdivision of the P2 receptor family and are activated by purine nucleotides such as ATP, GTP and, to a lesser extent, by related pyrimidine nucleotides such as CTP and UTP. Another subdivision of P2 receptors is represented by the G protein-coupled P2Y receptors, of which there are eight members – $P2Y_{1,2,4,6,11,12,13,14}$ receptors – activated by purine or pyrimidine nucleotides (and sometimes both). The function of P2Y receptors in polarized epithelial cells has been discussed in an earlier review [1].

Seven genes encode the P2X receptor subunits in mammals; otherwise, simpler vertebrates (e.g., zebra fish) may possess up to nine genes. Examples of P2X receptor genes have also been found in invertebrates [2]. In heterologous expression systems, the seven mammalian subunits form functional homomeric assemblies (called $P2X_{1-7}$; fig. 1). Seven functional heteromeric assemblies formed by the union of two different P2X subunits ($P2X_{1/2,1/4,1/5,2/3,2/6,4/6,4/7}$) have also been established from a larger cohort of 13 heteromeric assemblies (that further includes $P2X_{1/3,1/6,2/5,3/5,4/5,5/6}$) first identified in immunoprecipitation studies of co-expressed subunits. Established P2X receptor subtypes have distinct pharmacological and operational profiles but, fundamentally, all are ATP receptors and all are potently activated by this energy molecule (fig. 1). There is a paucity of selective agonists and antagonists for each of the known P2X receptor subtypes and, accordingly, establishing a correspondence between native P2X receptors and known recombinant P2X receptor subtypes is, in equal measure, arduous and laborious. Such studies typically require the careful cross-correlation of the potency and efficacy of agonists at native and recombinant P2X receptors, their modulation by a range of substances and by the capacity of a limited number of antagonists to block P2X receptor subtypes (table 1).

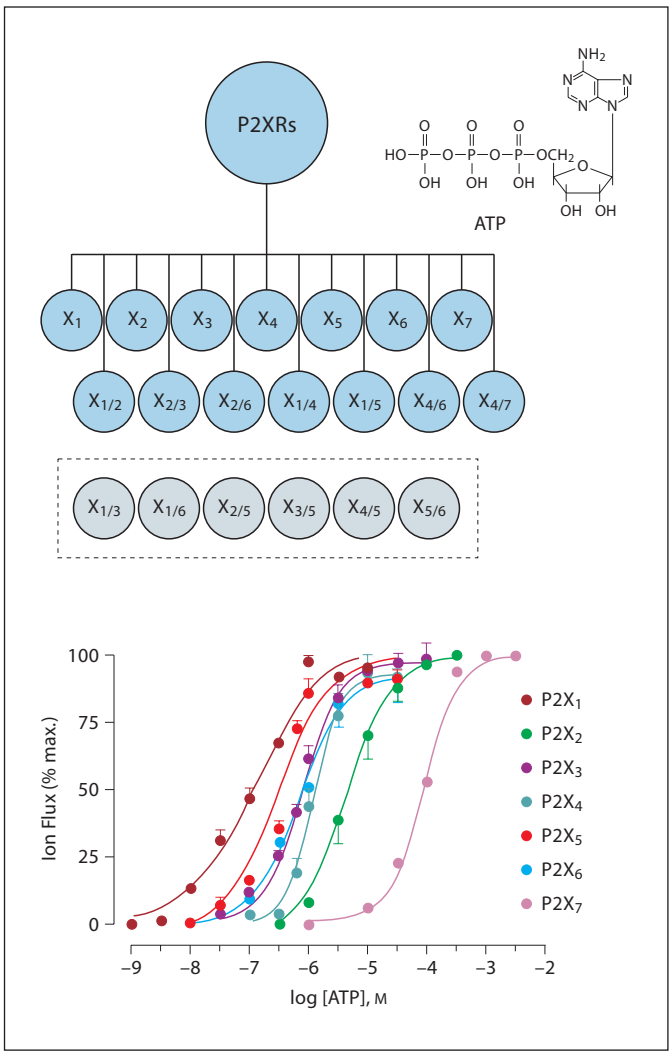

Fig. 1. P2X receptors. The P2X receptors comprise a series of seven homomeric assemblies ($P2X_1$–$P2X_7$) and a second series of seven heteromeric assemblies ($P2X_{1/2}$, $P2X_{1/4}$, $P2X_{1/5}$, $P2X_{2/3}$, $P2X_{2/6}$, $P2X_{4/6}$ and $P2X_{4/7}$; dark blue circles). A further series of heteromeric assemblies has been identified from immunoprecipitation studies, but none has yet been established as functional ion channels (pale blue circles). The series of $P2X_1$–$P2X_7$ receptors have different affinities for ATP which, as shown in the accompanying graph, is more potent at certain assemblies. The potency indices for ATP are given in table 1.

It is generally accepted that P2X receptor assemblies are comprised of 3 glycosylated subunits [2]. Accordingly, three ATP molecules are required for receptor activation. Each subunit protein has two transmembrane-spanning domains, with intracellular N and C termini and a highly folded extracellular loop held together by a number of disulfide bridges. The binding sites for ATP are thought

Table 1. Homomeric P2X receptor subtypes

	$P2X_1$	$P2X_2$	$P2X_3$	$P2X_4$	$P2X_5$	$P2X_6$	$P2X_7$
Gene (human)	*17p13*	*12q24.33*	*11q12*	*12q24.32*	*17p13*	*22q11.21*	*12q24.31*
Protein (rat)	399 aa	472 aa	397 aa	388 aa	445 aa	379 aa	595 aa
Natural ligands (pEC_{50})	ATP (7.0) CTP (4.4) Ap_6A (6.0) Ap_6G (5.7)	ATP (5.3) Ap_4A (4.8)	ATP (5.9) UTP (4.0) Ap_6A (5.9) Ap_3A (6.0)	ATP (5.4) CTP (3.5) Ap_4A (5.5)[a]	ATP (6.4) GTP (4.6) Ap_4A (6.6)	ATP (6.3)	ATP (3.4) ADP (2.7) (m)[b]
Other agonists (pEC_{50})	2MeSATP (7.0) ATPγS (6.2) αβmeATP (5.5) BzATP (4.6)	2MeSATP (5.1) ATPγS (5.1) αβmeATP (3.0)	2MeSATP (6.7) ATPγS (5.9) αβmeATP (5.7) BzATP (7.1)	2MeSATP (3.6) ATPγS (~5.0) αβmeATP (4.2)[a]	2MeSATP (6.4) ATPγS (6.5) αβmeATP (6.0) BzATP (5.9)	αβmeATP (6.2)	2MeSATP (5.0) BzATP (5.2)
Antagonist – selective (pIC_{50})	NF449 (9.5) Ip_5I (8.5)		A317491 (7.6)				A740003 (7.75) BBG (8.0) KN-62 (7.4) (h)[b]
Antagonist – nonselective (pIC_{50})	TNP-ATP (9.0) PPADS (6.9) Suramin (5.7) RB-2 (5.7)	TNP-ATP (5.9) PPADS (5.8) Suramin (5.0) RB-2 (6.4)	TNP-ATP (9.5) PPADS (6.7) Suramin (5.4) RB-2 (4.3)	TNP-ATP (4.8) PPADS (<4.0) Suramin (<4.0) BBG (3.9)	TNP-ATP (6.3) PPADS (6.7) Suramin (5.8) RB-2 (4.7)	TNP-ATP (6.1) PPADS (4.7)	TNP-ATP (~4.3) PPADS (4.3) Suramin (<4.0)
Signaling	Cation channel P_{Ca}/P_{Na} ~4	Cation channel P_{Ca}/P_{Na} ~2	Cation channel P_{Ca}/P_{Na} ~4	Cation channel P_{Ca}/P_{Na} ~4	Cation channel P_{Ca}/P_{Na} ~1.5	Cation channel ND	Channel-to-pore Pass size <700 Da

The pharmacological and signaling properties of homomeric P2X receptors, listing the natural and synthetic agonists and selective and nonselective antagonists for each P2X receptor subtype. Data are given as $-\log_{10}$ EC_{50} (pEC_{50}) and $-\log_{10}$ IC_{50} (pIC_{50}). Data are given for rat isoforms. Adapted from Bailey et al. [35].

[a] Data are given for partial agonists in some cases.

[b] Data are also given for human (h) and mouse (m) isoforms in a few cases.

to lie between (rather than within) the extracellular loops. The integral ion channel formed by the close association of six transmembrane-spanning domains (i.e., two from each of three subunits) is permeable to Na^+,K^+ and Ca^{2+} ions and, in a few examples, also to Cl^- ions. Upon activation, P2X receptors open within milliseconds and cause cell depolarization by a predominant cationic influx. Via Ca^{2+} influx, P2X receptors also activate intracellular signaling cascades (including transcription pathways) and can regulate the activity of a broad series of intracellular kinases [3]. Ca^{2+} influx makes up some 3–15% of the total current carried by P2X receptor ion channels [4].

The current carrying capacity differs between the known P2X receptor subtypes. Some (including $P2X_1$, $P2X_{1/2}$, $P2X_{1/5}$ and $P2X_3$) activate, then rapidly inactivate, in the continued presence of extracellular ATP. These P2X subtypes can produce membrane currents that are large in amplitude yet brief in duration, and they are referred to as 'rapidly desensitizing' P2X receptors. They may serve a role for intermittent, yet intense, cell signaling. Others (including $P2X_2$, $P2X_{2/6}$, $P2X_4$ and $P2X_{4/6}$) activate, then slowly inactivate, over a longer period of time (10–100 s), and these are referred to as 'slowly desensitizing' P2X receptors. Such P2X subtypes may play a role in prolonged, and probably tonic, cell signaling. Lastly, the activated $P2X_7$ receptor, in sharp contrast to all other P2X subtypes, progresses to a non-inactivating conductance state that involves the opening of an ancillary membrane channel [5]. This may bring about profound changes to the cell and may limit its lifespan.

There are also striking differences in the amplitude of currents carried by P2X receptors, a phenomenon related both to receptor number and the efficiency of receptor insertion into the surface membrane. Homomeric $P2X_5$ and $P2X_6$ receptors carry only small amplitude membrane currents, because these assemblies traffic poorly to the cell surface and numbers there are low. On the other hand, $P2X_1$, $P2X_2$, $P2X_3$ and $P2X_4$ receptors yield large amplitude membrane currents because they are transported more efficiently to the cell surface. Of note, some P2X subtypes ($P2X_2$, $P2X_{2/3}$, $P2X_4$, $P2X_5$ and $P2X_7$) undergo a time-dependent change in ion channel con-

ductance that alters the pass size of ion channel permeants – a phenomenon called pore dilatation. $P2X_7$ receptors first undergo pore dilatation and, subsequently, activate a much larger ancillary pore [5].

P2X Receptor Distribution

Immunohistochemical, RT-PCR and pharmacological studies have demonstrated the presence of P2X receptors in all types of cells, including neurons, glia, muscle, bone and hemopoietic tissues, endothelial and epithelial cells [6]. In spite of the widespread distribution of P2X subunits in cells and tissues, much of the research on P2X receptors has focused on just a few key roles, such as smooth muscle contraction (involving PX_1 and $P2X_5$ subunits), neuronal excitation (involving $P2X_2$, $P2X_3$, $P2X_4$ and $P2X_6$ subunits), inflammation and cell death (involving $P2X_7$ subunits). With gene targeting in mice, P2X receptor subtypes have been linked to less traditional roles such as autoregulation of renal blood flow ($P2X_1^{-/-}$ data), to pain, visceral sensation, ventilatory responses during hypoxia ($P2X_2^{-/-}$ and $P2X_3^{-/-}$ data), bone growth and macrophage function ($P2X_7^{-/-}$ data) [7].

Investigators have reported the surface expression of nearly all P2X (and P2Y) proteins in epithelial cells of the kidney, lung, gastrointestinal tract and accessory exocrine glands [8–11]. P2X (and P2Y) receptors occur on the apical and basolateral membranes of native epithelial cells and related cell lines, with some evidence for polarity in their distribution [12]. Investigators have also shown that ATP can be released from epithelial cells in vitro by a series of stimuli such as membrane distortion, osmotic swelling, hypoxia and acidosis [13]. ATP extrusion pathways have been linked to the CFTR protein, to P-glycoprotein-based organic anion transporters, stretch-activated cationic channels (and Ca^{2+}-dependent exocytosis of ATP) and also to hemiconnexin channels [7, 14]. In turn, regulated ATP release has been linked to disparate cell functions, such as cell defense, hydration of the mucus barrier, enhanced ciliary beating, cell volume regulation, ischemic protection and cell division [10].

P2X Receptors in the Kidney

All seven P2X subunits have been localized by immunohistochemical techniques in kidney tissue, with identifiable patterns of distribution along the rat nephron [11, 15, 16]. $P2X_{1-3}$ subunits have been found mainly in vascular smooth muscle, while $P2X_2$ and $P2X_7$ subunits were found in endothelial and mesangial cells, as well as podocytes. High levels of $P2X_4$ and $P2X_6$ proteins occur in renal tubule cells, sometimes accompanied by relatively low levels of $P2X_1$, $P2X_2$, $P2X_5$ and $P2X_7$ protein. The variety and abundance of P2X subunits is greatest in the distal segments of the renal tubule, with $P2X_{1,2,4,5,6,7}$ subunits present in the apical membrane of collecting duct (CD) principal cells in the rat, and $P2X_4$, $P2X_6$ and $P2X_7$ subunits in the basolateral membrane. Using real-time PCR, mRNA levels in the CD are highest for $P2X_4$, with $P2X_6$ some 10-fold lower [16]. Transcripts for $P2X_1$, $P2X_2$ and $P2X_4$ are found in similar quantities as $P2X_6$ in the distal tubule. In salt-depleted rats (maintained on 0.01% NaCl for 8–10 days), there is a significant upregulation of $P2X_1$, $P2X_4$ and $P2X_6$ transcripts in CD cells [16].

With respect to the vasculature, activation of P2X receptors typically causes vasoconstriction. This applies especially to the afferent arteriole, where ATP in the interstitium and probably originating from the macula densa can profoundly regulate glomerular perfusion and intraglomerular pressure. With gene deletion in $P2X_1^{-/-}$ mice, this effect is largely ablated [17]. Perfusion of the renal vasculature with nucleotides results in both short- and long-lasting increases in the muscle tone of blood vessels. This dual action points to heterogeneity amongst the endogenous P2X receptors. By itself, the rapidly desensitizing $P2X_1$ cannot comfortably explain the long-term actions of perfused nucleotides and, more likely, a mixture of homomeric and heteromeric P2X receptors are involved. The molecular identity of P2X receptors responsible for tonic vasoconstriction is a key area for future research.

In the nephron, nucleotides in the luminal fluid may reach sufficiently high concentrations to activate P2X receptors on the apical membrane [18]. At one time, it was suggested that luminal nucleotides took their origin from the blood and were filtered freely by the glomerulus, thereafter to be concentrated in the tubular fluid. However, kidney micropuncture studies support an alternative hypothesis that principally involves ATP secretion in the proximal convoluted tubule with a minimal contribution towards the total ATP concentration by glomerular filtration [19]. The molecular basis of tubular ATP secretion is presently unknown, but mechanoreception via the central cilium may play a key role here [20].

For a number of reasons, most reports on the regulation of ion transport by extracellular ATP have focused on the involvement of P2Y receptors, to the exclusion P2X receptors. Traditionally, signaling by P2X receptors has

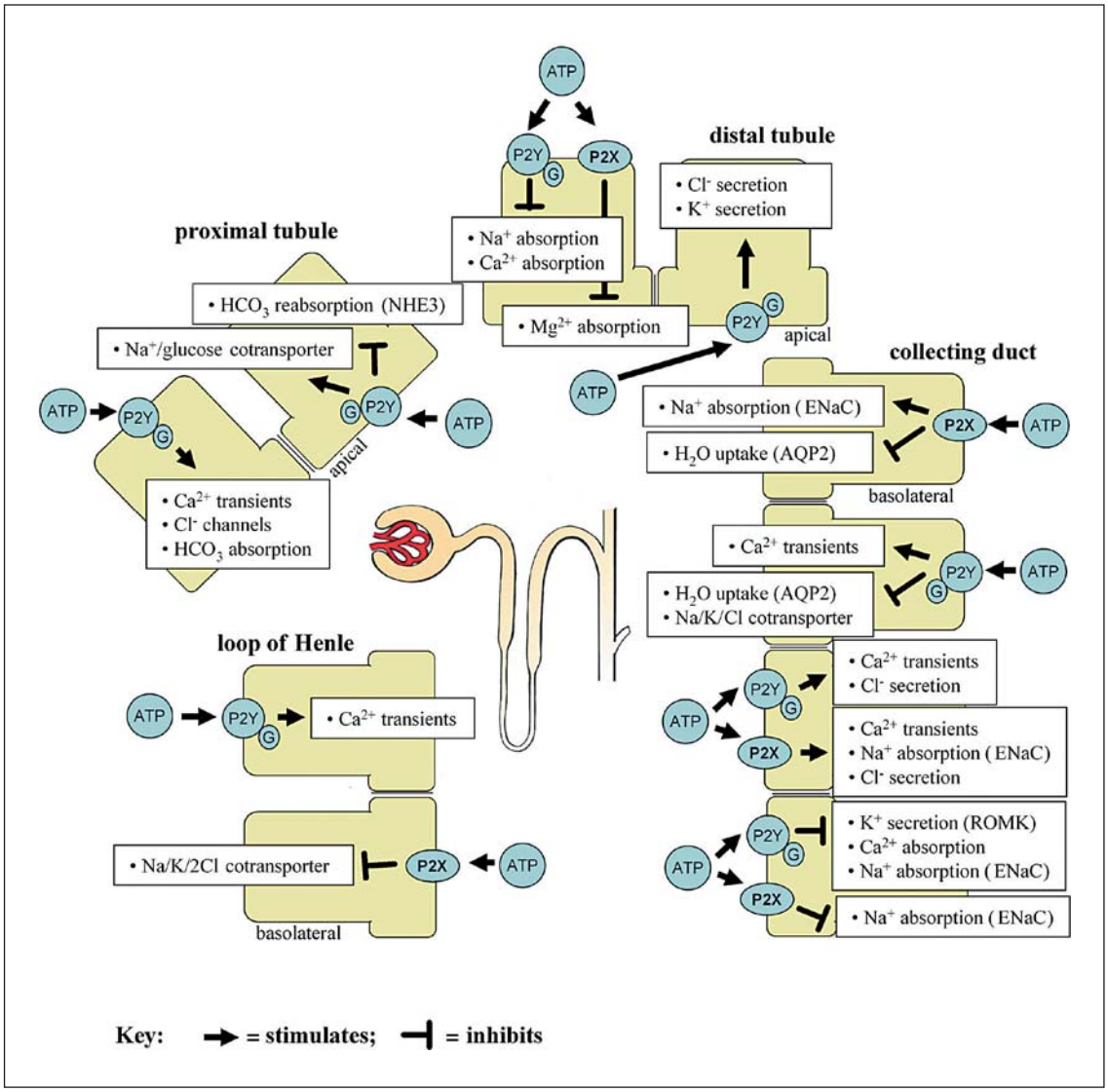

Fig. 2. Extracellular ATP actions in renal epithelium. A summary of effects of extracellular ATP in the nephron and epithelial cell lines derived from specific nephron segments [15, 16, 27, 29, 32]. In most cases, the activated P2R subtypes have not been identified. Many of the effects evoked by extracellular ATP in distal nephron epithelia are attributed to the activation of P2X receptors.

been associated with short-term phenomena and, accordingly, transient effects have been considered to have no practicality in the renal tubule. P2Y receptors, on the other hand, appear to be abundant in the apical membrane of the renal tubule, especially $P2Y_2$-like receptors in mouse tissues and mouse-derived cell lines which are used commonly in renal research. Consistently, signaling by $P2Y_2$ receptors has been associated with profound and long-term actions wherever they are found. For example, $P2Y_2$ receptors have been associated with cell growth in epithelial and non-epithelial cells and, in part, found to exert their effects genomically. However, our immunohistochemical data (unpublished) from rat and human renal tubules place $P2Y_2$ receptors primarily on intercalated cells of the CD [11, 16] or the basolateral membrane of tubules (unpublished). We can say with some authority that, instead, $P2Y_4$ receptors appear in the apical membrane of principal cells of rat CDs [16]. However, $P2Y_2$ and $P2Y_4$ receptors are almost identical in their pharmacological profiles as far as rodent isoforms are concerned

[21, 22] and it is easy to see why $P2Y_2$ receptors were implicated in past studies. The involvement of apical $P2Y_2$ receptors in regulating kidney function probably has been overrepresented through misguided pharmacology. The actions of extracellular ATP in the renal epithelium are listed in figure 2.

Many of the effects evoked by extracellular ATP in the distal nephron (and derived cell lines) can be attributed to the activation of P2X receptors, particularly where transport of Mg^{2+}, Na^+ and Cl^- ions is concerned [23–27] and movement of water is considered [28, 29]. For Mg^{2+} transport, P2X receptor activation in a mouse distal convoluted tubule cell line resulted in the inhibition of magnesium absorption by a presumed action on TRPM6 channels [24]. This inhibition occurred via an increase in intracellular Ca^{2+} ions which may enter through as yet unidentified P2X receptors involving $P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$ and $P2X_5$ subunit proteins which were found in this cell line [24]. Similarly, P2X-mediated calcium influx involving $P2X_1$ and/or $P2X_4$ subunits has been reported in isolated perfused mouse medullary CD preparation [27]. Further to these observations, it should be noted that $P2X_1$ subunits are capable of polymerization with $P2X_2$, $P2X_3$, $P2X_4$ and $P2X_5$ subunits, to form a number of subtypes of P2X receptors.

For Na^+ transport, tubular perfusion of ATP inhibits Na^+ uptake in the isolated mouse CCD even though ENaC channels are insensitive to nucleotides [30]. Several independent studies link this phenomenon in part to P2X receptor activation. First, Na^+ absorption and Cl^- secretion appears to involve $P2X_3$ and $P2X_4$ receptors in mIMCD-K2 cells, an immortalized mouse CD cell line [23]. Second, the recovery of $^{22}Na^+$ ions in the urine of Na^+-restricted living rats is increased in distal tubules microperfused with the nonhydrolysable nucleotide, ATPγS [25]. This effect was linked to the activation of $P2X_{4/6}$ receptors [31]. Third, the sequential co-expression of rat ENaC channels with rat P2X receptors subtypes in *Xenopus* oocytes showed that a single ATP challenge irreversibly inhibited ENaC channels when either $P2X_2$, $P2X_4$, $P2X_{2/6}$ or $P2X_{4/6}$ receptors were activated [28]. Inhibition occurred by trafficking ENaC subunits out of the cell membrane and, mechanistically, was associated with Na influx through $P2X_2$ and $P2X_{2/6}$ receptors and Na^+ and Ca^{2+} influx through $P2X_4$ and $P2X_{4/6}$ receptors. Recently, patch clamp experiments in the microdissected CD of the rat kidney showed that nucleotide activation of $P2X_4$ and possibly $P2X_{4/6}$ receptors on the apical surface of principal cells in situ can either inhibit or potentiate an amiloride-sensitive inward current, presumably involving ENaC channels, depending on the concentration of extracellular sodium [16]. These extended observations have led to the proposal that $P2X_4$ and $P2X_{4/6}$ receptors may function as sodium sensors responsible for local regulation of ENaC activity in the rat CD in response to changes in luminal sodium concentration. Potentiation of ENaC-mediated currents by $P2X_4$ receptors, albeit those expressed on the basolateral membrane, has also been reported in A6 cells [32].

For the movement of water, sequential co-expression of rat AQP2 channels with known rat P2X receptor subtypes in *Xenopus* oocytes showed a diminished responsiveness in a swelling assay, but only when $P2X_2$ receptors were present, and they were activated by ATP [28]. Furthermore, dDAVP treatment of cultured mpkCCD(c14) cells not only increased AQP2-positive immunostaining, but also $P2X_1$ and $P2X_2$ subunit immunostaining in the apical membrane; secondly, activation of these apical P2X receptors with ATPγS removed immunopositive AQP2 protein from the apical membrane [29]. This apart, $P2Y_2$ receptors on the basolateral membrane of principal cells are also known to regulate water movement in the CD.

Are P2X Receptors Also Directly Involved in the Net Transport of Solutes?

It is not unreasonable to suppose that, as apical membrane channels, P2X receptors can assist in transporting sodium and calcium across the kidney tubule. We have calculated the charge transfer during the maximal activation of $P2X_2$ receptors and estimated the net increase in intracellular sodium to be 0.5 μmol/min for a model cell (*Xenopus* oocyte, 1 μl cell volume) [26]. We have calculated the charge transfer for activated ENaC channels, which yielded tonic inward currents of comparable amplitude to that evoked by $P2X_2$ receptors, and estimated the net increase in intracellular sodium to be 5 μmol/min. Even if channel numbers were matched in vivo, the lower amount of sodium carried by $P2X_2$ receptors compared to ENaC channels suggests a minor role, at best, for the former in sodium transport.

P2X Receptors as Regulators of Other LGICs

Functional interactions between P2X receptors and other LGICs have been described in excitable tissues and in expression systems [2]. Thus far, cross-inhibition ap-

pears to be the norm when P2X receptors are co-expressed, or co-localized, with members of the Cys-loop superfamily of LGICs including $GABA_A$, $GABA_C$, nACh and 5-HT_3 receptors. Use of fluorescence resonance energy transfer and total internal reflection fluorescence suggests that $P2X_2$ receptors and nACh ($\alpha4\beta2$) receptors physically interact and the activation of one can lead to the internalization of both. As indicated above, it has also been postulated that TRPM6 channels are also regulated by P2X receptors, but the mechanism behind this regulation is unknown [24]. Otherwise, TRPV1 was shown to exert a one-way inhibition on $P2X_{2/3}$ and $P2X_3$ receptors again by an unknown mechanism [33, 34]. As yet, it remains to be shown how and where channel–channel interactions occur naturally in the kidney tubule.

Conclusions

For the kidney, the role of P2X receptors in polarized epithelial cells has been largely neglected. However, our research on kidney cell function over the past 5 years, including our supporting work of modeling in expression systems, now points to a regulatory role of P2X receptors in the movement of salt and water across the wall of the distal tubule and CDs. We find no credible evidence for a role of P2X receptors in punctate cell signaling, which is the conventional role for P2X receptors outside the kidney and in excitable tissues. Instead, P2X receptor activation and resultant cationic currents appear to change the rate of internalization and reclamation of salt and water channels. Uppermost in our minds is the question of the physiological relevance of these phenomena. Nature, typically, is economical; complex mechanisms tend to evolve into efficient regulatory processes rather than linger as redundant systems. Thus, we are working towards a better understanding of this regulatory mechanism and, in writing this review, we hope that our opinions will stimulate a debate and lead to new research directions.

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