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P2Y receptors and kidney function

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Abstract

Cellular release of nucleotides is of physiological importance to regulate and maintain cell function and integrity. Also in the tubular and collecting duct system of the kidney, nucleotides are released in response to changes in cell volume or luminal flow rate and act in a paracrine and autocrine way on basolateral and luminal P2Y receptors. Recent studies using gene knockout mice assigned a prominent role to G protein-coupled P2Y₂ receptors, which are activated by both ATP and UTP. The antidiuretic hormone, arginine-vasopressin (AVP), and possibly an increase in collecting duct cell volume induce ATP release. The subsequent activation of P2Y₂ receptors inhibits AVP-induced cAMP formation and water reabsorption, which stabilizes cell volume and facilitates water excretion. An increase in NaCl intake enhances luminal release of ATP and UTP in the aldosterone-sensitive distal nephron which by activating apical P2Y₂ receptors and phospholipase C lowers the open probability of the epithelial sodium channel ENaC, thereby facilitating sodium excretion. Thus, the renal ATP/UTP/P2Y₂ receptor system not only serves to preserve cell volume and integrity but is also regulated by stimuli that derive from body NaCl homeostasis. The system also inhibits ENaC activity during aldosterone escape, i.e. when sodium reabsorption via ENaC is inappropriately high. The P2Y₂ receptor tone inhibits the expression and activity of the Na-K-2Cl cotransporter NKCC2 in the thick ascending limb and mediates vasodilation. While the role of other P2Y receptors in the kidney is less clear, the ATP/UTP/P2Y₂ receptor system regulates NaCl and water homeostasis and blood pressure.

Autocrine and paracrine mechanisms are important for the regulation of renal function and to maintain the integrity of kidney cells. Cellular release of ATP and other nucleotides constitute such local intrarenal mechanisms. The nucleotide release in renal epithelia occurs across both the apical and basolateral membranes with a proposed predominant release across the apical membrane^{1–4}. Cell membrane ecto-nucleotidases degrade extracellular nucleotides, and the expression pattern of these enzymes along the nephron and collecting duct (CD) system may provide functional clues^{1;5;6}. The released ATP and other nucleotides affect cellular function by activating ionotropic P2X receptors and/or metabotropic G protein-coupled P2Y receptors in the cell membrane. The P2Y receptor family currently includes eight receptor subtypes (P2Y_{1,2,4,6,11–14})^{7–9} and their role in the

kidney is the focus of this review. The recent availability of gene knockout animals has provided an invaluable tool to better define the function and relevance of P2Y receptors in the kidney. These studies, in particular, point to a prominent role for the P2Y₂ receptor in the regulation of renal electrolyte and water transport. Where evidence is available for a functional role of other P2Y receptor subtypes, this will be mentioned. For a more detailed discussion, the interested reader is referred to recent reviews on the role of P2Y receptors in the kidney^{1–3;10–13}.

EXPRESSION AND FUNCTION OF P2Y RECEPTORS IN RENAL VASCULATURE

Expression data of P2Y receptors in the renal vasculature are sparse compared to other parts of the kidney. One study proposed expression of P2Y₁ and P2Y₂ receptor in the smooth muscle cells of rat renal artery¹⁴, another study proposed that afferent and efferent arterioles express P2Y₁ receptors¹⁵. Little is known about the specific role of P2Y receptor subtypes beyond the general notion that the sensitivity of the renal vasculature appears to be greater to P2X receptor stimulation than P2Y under basal conditions, and that P2X receptor stimulation causes vasoconstriction, whereas activation of P2Y receptors in the kidney causes vasoconstriction or tone-dependent vasodilation (for review see¹⁶). Studies in the blood perfused juxtamedullary nephron indicated afferent arteriolar vasoconstriction when P2Y-selective agonists like 2-methylthio ATP (preferred acting on P2Y₁) and UTP (P2Y₂ and/or P2Y₄) were applied¹⁷. ATP, UTP and 2-methylthio ATP caused a dose-dependent vasoconstriction in isolated rat renal arteries pre-contracted with KCl¹⁴, a maneuver that has been shown to impair endothelial-dependent vasorelaxation^{18;19}. In the isolated perfused rat kidney 2-methylthio ATP, ADP (P2Y_{1/12}), ATP γ S (P2Y₂) and UTP produced vasodilatation at low doses and vasoconstriction at high doses²⁰. The vasodilation response appeared to be mediated by an endothelial factor, including nitric oxide (NO) and endothelial-derived relaxation factor (EDHF), and independent of prostaglandin formation^{20–22}. Indirect in vivo evidence for a tonic vasodilatory role of P2Y₂ receptor activation in the kidney comes from P2Y₂ receptor knockout mice (P2Y₂ $-/-$) which show an increased renal vascular resistance compared to wild-type mice (WT)²³. Systemic application of a P2Y_{2/4} receptor agonist (INS45973) dose-dependently decreased blood pressure in wild-type mice and to a similar extent in endothelial nitric oxide synthase knockout mice²⁴. By contrast, INS45973 increased blood pressure in P2Y₂ $-/-$ mice. These studies indicated that P2Y₂ receptor activation induces a systemic vasodilation that is independent of endothelial NO synthase, implicating a role for EDHF, and that activation of P2Y₄ receptors on smooth muscle may induce vasoconstriction²⁴. Figure 1 proposes a general concept for the role of P2Y receptors in the regulation of vascular tone, which may in part apply to the renal resistance vessels. Much more information is required on the role of P2Y receptors on the afferent versus efferent arteriole, the glomerular capillaries, and the postglomerular cortical and medullary vasculature.

EXPRESSION OF P2Y RECEPTORS IN RENAL EPITHELIA

Table 1 summarizes the renal epithelial expression of P2Y receptors based on analysis of mRNA and protein expression in rat kidney. Defining the functional role of specific P2Y receptor subtypes has been hampered by the lack of receptor subtype specific antagonists. First and preliminary information on the role of a specific P2Y receptor subtype has been obtained based on their differences in agonist preference, e.g., P2Y₆ receptors are activated by UDP>UTP>ADP; in rodents only the P2Y₂ and P2Y₄ receptor subtypes are similarly activated by ATP and UTP^{8;9} and named “P2Y₂-like” in the following. Table 2 summarizes the latter kind of studies that have been performed on the proximal tubule. These studies propose a potential role of P2Y₂-like receptors in the regulation of gluconeogenesis in the

proximal tubule, but further studies are needed to identify the receptor subtype and the physiological relevance.

FLOW-DEPENDENT NUCLEOTIDE RELEASE AND ACTIVATION OF P2Y₂ RECEPTORS IN THICK ASCENDING LIMB

Binding studies^{25,26} and studies of the effects of ATP and UTP on $[Ca^{2+}]_i$ ²⁷ provided evidence for the presence of P2Y₂-like receptors in thick ascending limb (TAL), i.e. P2Y₂ and/or P2Y₄ receptors. Both receptors have been detected in TAL on the mRNA and/or protein level (Table 1). Studies in perfused medullary TAL of WT and knockout mice indicated that increasing the perfusion pressure and thereby the luminal flow rate induces apical and basolateral release of nucleotides, which by activation of apical and basolateral P2Y₂ receptors triggers increases in $[Ca^{2+}]_i$ ²⁸ and $[Ca^{2+}]_i$ oscillations²⁹. First evidence for an inhibitory influence of P2Y₂ receptor tone on NaCl reabsorption in TAL was provided in P2Y₂^{-/-} mice which have greater expression of NKCC2^{23,30} and furosemide-induced natriuresis compared with WT mice²³. The ATP/UTP/P2Y₂ receptor system may serve to inhibit and limit NaCl reabsorption in TAL, which may support and interact with basolateral P2X receptors³¹ and the adenosine system³² in the metabolic control of TAL function.

THE ATP/UTP/P2Y₂ RECEPTOR SYSTEM INHIBITS THE EPITHELIAL SODIUM CHANNEL

The expression of P2Y₂ receptor protein has been demonstrated in both apical and basolateral membranes of rat collecting ducts (CD) (Table 1). Studies in the isolated perfused rabbit and mouse cortical collecting duct (CCD) indicated the expression of both basolateral and luminal P2Y₂-like receptors in principal cells^{33–35}. These receptors are linked to activation of phospholipase C and increases in $[Ca^{2+}]_i$ ^{33–35}. In 1996, experiments in cultured rabbit connecting tubule (CNT) and CCD cells provided first evidence for an inhibitory effect of ATP on sodium transport via the epithelial sodium channel, ENaC, an effect that appeared to be independent of changes in $[Ca^{2+}]_i$ ³⁶. Subsequent studies in M-1 mouse CCD cells^{37,38} and in isolated perfused CCD of mice kept on a low NaCl diet³⁹ indicated that both luminal and basolateral activation of P2Y₂-like receptors can inhibit ENaC-mediated Na⁺ reabsorption independent of an increase in $[Ca^{2+}]_i$.

Activation of P2Y₂ receptors reduces ENaC open probability

The initial *in vivo* evidence for an inhibitory effect of luminal ATP on Na⁺ reabsorption in the CD was obtained in 2005 in micropfusion experiments in rats maintained on a low Na⁺ diet⁴⁰. In 2007, studies in P2Y₂^{-/-} mice revealed normal amiloride-sensitive Na⁺ reabsorption despite lower renal expression of the α -subunit of ENaC compared with WT mice²³, indicating greater intrinsic activity of ENaC. Moreover, smaller increases in plasma aldosterone were sufficient to adapt renal Na⁺ excretion to restricted dietary NaCl intake in P2Y₂^{-/-} mice²³, a finding that could also be explained by greater intrinsic ENaC activity. Subsequent patch-clamp studies in freshly isolated split open CNTs and CCDs of knockout and WT mice demonstrated that locally released ATP acts in an autocrine/paracrine manner to tonically inhibit the open probability (P_O) of ENaC via activation of P2Y₂ receptors^{41,42} (Figure 2). Thus, a lack of P2Y₂ receptor activation in mice resulted in greater ENaC P_O . The latter was in part compensated by decreasing circulating aldosterone concentrations and ENaC expression to normalize net ENaC activity.

Inhibition of ENaC open probability by P2Y₂ receptors involves activation of phospholipase C and hydrolysis of anionic phospholipids

In the A6 cell line, the P2Y receptor agonist ATP γ S reduced ENaC P_O by activation of phospholipase C⁴³ and hydrolysis of the anionic phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP₂)⁴⁴. Studies in mpkCCDc14 principal cells showed that ATP decreases plasma membrane PIP₂ levels and ENaC P_O with similar time courses⁴⁵. Moreover, direct activation of PLC mimicked ATP action on ENaC in isolated CNT/CCD, and direct PLC stimulation decreased ENaC activity in CNT/CCD from P2Y₂^{-/-} mice⁴¹. Whole-cell patch clamp studies of principal cells of split-open CD from sodium-restricted rats confirmed that activation of P2 receptors (most likely the P2Y₂ and/or P2Y₄ subtype) inhibited ENaC activity via activation of PLC⁴⁶. Experiments in *Xenopus oocytes* and M-1 mouse CCD cells⁴⁷ as well as A6 cells⁴⁸ provided further evidence for the following mechanism for the regulation of ENaC by P2Y₂ receptors⁴⁹ (Figure 3): under resting conditions the inner leaflet of the lipid bilayer contains a high concentration of PIP₂, the latter binding the N-terminus of the β -subunit of ENaC, thereby maintaining the ENaC channel open. Stimulation of P2Y₂ receptors activates phospholipase C, which hydrolyzes and lowers the concentration of PIP₂ with resultant decreases in PIP₂ binding to the N-terminus of β -ENaC. The latter reduces ENaC activity by lowering P_O .

Dietary NaCl inhibits the open probability of ENaC in connecting tubule/cortical collecting duct by enhancing apical P2Y₂ receptor tone

Patch-clamp studies in freshly isolated mouse CNT/CCD demonstrated that ENaC P_O is negatively regulated by dietary NaCl^{42;50}. Gene-knockout of P2Y₂ receptors in mice did not affect the inhibitory effect of dietary NaCl on the number of ENaCs per patch (N) but completely prevented the dietary NaCl-induced lowering of ENaC P_O (Figure 2)⁴². Inhibition of apical ATP/P2Y-receptor signaling (using suramin to block the receptors and hexokinase/glucose to remove ATP) in WT mice prevented the inhibitory influence of dietary NaCl intake on ENaC P_O (and mimicked the phenotype of P2Y₂^{-/-} mice), consistent with a dietary NaCl dependent release of ATP/UTP from CNT/CCD that is preserved after harvesting these segments (Figure 2)⁴². In accordance, a greater NaCl intake was associated with increased urinary concentrations (relative to creatinine) of UTP⁴², the ATP hydrolytic product, ADP⁴², and ATP⁵¹. Moreover, the urinary concentrations of UTP and ATP were within the range of concentrations shown to alter ENaC activity in freshly isolated split open CNT/CCD⁵¹.

THE RELEVANCE OF THE ATP/UTP/P2Y₂ RECEPTOR SYSTEM FOR NaCl HOMEOSTASIS AND BLOOD PRESSURE REGULATION

Deleting P2Y₂ receptors in mice caused a NaCl-resistant increase in BP²³. A dysfunction of arterial baroreceptors has been implicated in genetic forms of NaCl-sensitive hypertension in rats and humans⁵². In comparison, it was proposed that the increased BP in P2Y₂^{-/-} mice is NaCl-resistant due to an intact baroreceptor response, which lowers heart rate and tends to normalize renal NaCl excretion by inhibiting the sympathetic nervous tone and the renin-angiotension-aldosterone system: changes in NaCl intake did not affect heart rate in WT mice but negatively regulated heart rate in P2Y₂^{-/-} mice²³. Moreover, plasma aldosterone concentrations were lower in P2Y₂^{-/-} compared with WT mice on low, normal and high NaCl intake²³.

P2Y₂^{-/-} mice have an increased BP but amiloride-sensitive renal sodium excretion is not significantly increased compared with WT mice on a regular NaCl diet²³, consistent with a similar net ENaC activity (NP_O) in isolated CNT/CCD patches harvested from P2Y₂^{-/-} and WT animals on normal NaCl diet⁴². Lower plasma aldosterone concentrations can explain

the lower renal α ENaC expression observed in $P2Y_2^{-/-}$ mice which may normalize ENaC activity in the presence of greater ENaC P_O . Greater BP despite “normal” net ENaC activity implies additional BP-related defects in $P2Y_2^{-/-}$ mice, consistent with a proposed greater expression and activity of NKCC2 in the TAL²³ and impaired vasodilation (see below).

Chronic application of DOCA plus high NaCl intake eliminated the differences in mineralocorticoid tone between $P2Y_2^{-/-}$ and WT mice, and unmasked greater net ENaC activity as well as NaCl-sensitivity of BP in knockout compared with WT mice⁴². Furthermore, DOCA sensitized the number of active ENaC expression in the apical membrane to a $P2Y_2$ receptor-mediated inhibitory influence, indicating that the influence of the ATP/UTP/ $P2Y_2$ receptor system on ENaC goes beyond the regulation of P_O ⁴². Thus, $P2Y_2$ receptor signaling also mediates the suppression of ENaC activity when sodium transport is activated by high levels of mineralocorticoids in combination with high NaCl intake as observed in aldosterone escape^{42;51}.

Mechanisms involved in the regulation of luminal ATP/UTP release by dietary NaCl

The molecular mechanisms involved in the regulation of luminal ATP/UTP concentrations following changes in NaCl intake may include changes in nucleotide secretion and breakdown. The gap-junction protein, connexin 30 (Cx30), which may function as a hemichannel, has recently been implicated in flow-induced release of ATP in murine CD⁵³, in NaCl-induced increases in urinary ATP/creatinine ratios⁵¹ and NaCl-induced suppression of ENaC P_O ⁵⁴, as well as in the normal DOCA escape of ENaC⁵⁴. In contrast to $Cx30^{-/-}$ mice, mice lacking $P2Y_2$ receptors have lost completely the down-regulation of ENaC P_O by dietary NaCl or the upregulation of ENaC P_O by DOCA. Further studies are needed to test whether this reflects other pathways of ATP release and whether Cx30 is also a determinant of UTP release. The renal expression of rabbit and murine Cx30 is mostly restricted to intercalated cells of the CNT/CCD whereas Cx30 was found throughout the luminal membrane continuous from the medullary TAL to the medullary CD system in the rat kidney⁵⁵ indicating species differences.

In addition to changes in cell volume and luminal flow rate, the local release and possibly breakdown/conversion of ATP/UTP in the CNT/CCD may be directly or indirectly under control of hormones involved in NaCl homeostasis. Lowering NaCl intake or treatment with DOCA increased ENaC P_O in isolated CNT/CCD in WT mice but not in $P2Y_2^{-/-}$ mice⁴². Moreover, mineralocorticoids enhance ENaC P_O in A6 cells^{56–58}. Therefore, we speculate that mineralocorticoids increase ENaC P_O by suppressing the inhibitory influence of the ATP/UTP/ $P2Y_2$ receptor system (Figure 3). During mineralocorticoid escape (e.g. DOCA plus high salt diet) ATP and/or UTP release may primarily be due to increases in cell volume^{2;59;60} as a consequence of DOCA-induced overactive sodium transport and/or increases in tubular flow rate.

$P2Y_2$ receptor activation also induces systemic vasodilation

Whereas $P2Y_2$ receptor knockout impaired renal NaCl excretion, acute activation of $P2Y_2$ receptors inhibited renal sodium reabsorption and increased excretion²⁴. Moreover, $P2Y_2$ receptor activation mediated ATP-evoked relaxation of the murine aorta⁶¹ and an endothelial NO-independent BP lowering effect (implicating a role for EDHF)²⁴ (Figure 1). Dual vascular and renal effects suggest the potential utility of $P2Y_2$ agonism in the treatment of hypertension, and early genetic studies have identified polymorphisms in the gene for the $P2Y_2$ receptor associated with essential hypertension in Japanese men⁶² and with BP in African Americans⁶³.

A ROLE FOR P2Y₂-LIKE RECEPTORS IN THE INHIBITION OF RENAL K⁺ SECRETION

Patch-clamp studies of the apical membrane in split-open mouse CCD indicated that activation of P2Y₂-like receptors inhibit the small-conductance K⁺ channel of principal cells⁶⁴. In contrast, *in vitro* studies have linked ATP and P2Y receptor activation to an increased activity of big-conductance Ca²⁺-activated K⁺ (BK) channels in intercalated cells^{65;66}. The BK channel is of particular interest since an increase in tubular flow can not only increase ATP release and P2Y₂ receptor activation²⁸ but stimulates K⁺ secretion via the BK channel in the CNT/CD, which may involve P2Y₂ receptor-induced increases in [Ca²⁺]_i^{66–69}. Mice lacking P2Y₂ receptors provided indirect evidence for facilitated renal K excretion on standard K⁺ diet²³, which would be consistent with the proposed inhibitory influence of P2Y₂-like receptors on the small conductance K⁺ channel in principal cells discussed above. Facilitation of urinary K⁺ excretion in P2Y₂^{–/–} versus WT mice dissipated in response to a high K⁺ diet²³, which may reflect increasing contributions of P2Y₂ receptor stimulated BK channels under these conditions.

P2Y₂ RECEPTOR ACTIVATION INHIBITS WATER TRANSPORT IN THE COLLECTING DUCT

The anti-diuretic hormone arginine-vasopressin (AVP) is the primary regulator of water reabsorption in the renal CD and critically involved in the regulation of water balance and maintenance of plasma osmolality. AVP acts on the CD through the Gs protein-coupled vasopressin V₂ receptor (V₂R) to stimulate adenylyl cyclase (AC; in particular AC6 in the inner medulla) and thus the synthesis of cAMP⁷⁰. cAMP activates protein kinase A (PKA), which phosphorylates the water channel aquaporin-2 (AQP2), thereby resulting in apical plasma membrane insertion/retrieval of AQP2⁷¹ (Figure 4). In the following we outline the evidence that the CD system releases nucleotides like ATP/UTP in response to changes in cell volume and that these nucleotides act locally, including the activation of P2Y₂ receptors, to modulate water transport and stabilize cell volume (for review see^{2;3;72;73}).

P2Y₂ receptor protein expression has been localized in both apical and basolateral membranes of inner medullary collecting duct (IMCD) cells (Table 1), where it is positively related to the status of hydration⁷⁴ and suppressed by chronic V₂R agonism⁷⁵. Several studies in rabbit and rat CCD, OMCD and IMCD used the application of ATP and/or UTP to provide evidence for a P2Y₂-like receptor that activates a PLC/Ca²⁺/PKC signaling pathway and stimulates the release of prostaglandin E₂ (PGE₂) by activation of cyclooxygenase 1 thereby inhibiting cAMP formation and AVP-stimulated osmotic water permeability^{33;76–81} (Figure 4). Studies in mpkCCD cells indicated that P2Y₂ receptors are translocated to the basolateral membrane in response to AVP and mediate the downregulation of AQP2-mediated water transport in response to basolateral ATP⁸².

P2Y₂^{–/–} mice have greater renal medullary expression of AQP2 compared with WT mice despite having similar urinary AVP concentrations²³. P2Y₂ receptors also appear to inhibit the medullary protein expression of AQP3 and the urea transporters UT-A1 and UT-A2⁸³ (Figures 4). Functional studies in knockout mice indicated that P2Y₂ receptor activation inhibits the AVP-induced cAMP-formation and water transport in the CD^{2;23;73}. Moreover, measuring urinary ATP excretion while manipulating water homeostasis in P2Y₂^{–/–} and WT mice (using water loading or V₂R blockade)²³ provided indirect evidence for a cell volume- and AVP-induced ATP release in CDs. The latter, through activation of P2Y₂ receptors, inhibits water reuptake, stabilizes CD cell volume, and accelerates water excretion during hypotonic water loading (Figures 4 and 5) (for more detailed discussion see^{2;73}) as

suggested for other epithelia and cells ² and consistent with studies showing AVP-stimulated nucleotide secretion in perfused mouse medullary TAL and CCD ⁸⁴.

Conclusion

Recent studies have established the physiological relevance of the luminal ATP/UTP/P2Y₂ receptor system for the regulation of ENaC open probability in the CNT/CCD in response to changes in dietary NaCl and the relevance of the system for NaCl homeostasis and blood pressure regulation. These findings further indicate that local nucleotide/P2 receptor systems not only serve to preserve cell volume and integrity but may be regulated by stimuli that derive from body homeostasis. The implication of Cx30 in the regulation of nucleotide release by dietary NaCl is an important step to better understand the molecular nature and regulation of renal epithelial nucleotide release pathways. We have obtained novel insights about the renal epithelial localization and function of some P2Y receptors under basal condition but know very little about their pathophysiological relevance ⁸⁵. We are only beginning to understand the role of P2Y receptor subtypes in the renal vasculature ⁸⁶ and renal nerves ⁸⁷. Less is known about the interactions between nucleotide and adenosine receptor-mediated signaling including the formation of receptor heteromultimers (e.g., for P2Y₂ and adenosine A₁ receptors 8;88) or between adrenergic and purinergic signaling ⁸⁹. Finally and to better define the functional role and therapeutic potential, specific and more stable P2Y receptor antagonists and agonists are very much needed.

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Figure 1. A proposed model for the regulation of vascular tone by P2Y receptors

Depicted are localizations and vascular responses to activation of P2Y₁ (activated by ATP, 2-methylthio ATP), P2Y₂ (ATP/UTP/INS45973) and P2Y₄ (ATP/UTP/INS45973) receptors. Activation of P2Y₁ receptors on endothelial cells induces endothelium-dependent vasodilation utilizing nitric oxide (NO) and endothelial derived hyperpolarization factor (EDHF). Activation of P2Y₄ receptors on endothelial cells lowers vascular tone through release of endothelial NO; in comparison, activation of P2Y₂ receptors on endothelial cells induces vasodilation independent of endothelial NO and may involve EDHF. Activation of smooth muscle P2Y₁ or P2Y₄ receptors results in vasoconstriction. Based on and modified from ²⁴.

Figure 2. The inhibitory effect of ATP and dietary NaCl on ENaC open probability (P_O) in the aldosterone-sensitive distal nephron is mediated by P2Y₂ receptor activation

A) Continuous current traces from cell-attached patches on principal cells (containing two ENaC's) before and after application of ATP (100 μM) in CNTs/CCDs harvested from WT and P2Y₂^{-/-} mice fed a regular 0.32% Na⁺ diet. Patches clamped to $-V_p = -60$ mV and inward Li⁺ currents through ENaC are downward. Dashed lines indicate the respective current levels; c denotes the closed state. Summary graphs of ENaC P_O changes in response to ATP are shown on the right. * $P < 0.05$ vs. WT. B) The suppression of ENaC P_O by dietary NaCl intake is absent in P2Y₂^{-/-} mice. * $P < 0.05$ vs. WT. C) Inhibition of local ATP signaling (hexokinase (+glucose) to degrade local ATP plus suramin to prevent P2 receptor activation) prevents regulation of ENaC P_O by dietary NaCl intake in WT mice. * $P < 0.05$ vs. WT control. Based on and modified from ⁴².

Figure 3. A proposed model for the inhibition of ENaC open probability (P_O) by dietary NaCl in the aldosterone-sensitive distal nephron

Low dietary NaCl intake suppresses luminal release of ATP and UTP which may relate to increased aldosterone levels. This prevents activation of apical P2Y₂ receptors on principal cells (PC). As a consequence, the inner leaflet of the lipid bilayer contains a high concentration of negatively-charged phosphatidylinositol 4,5-bisphosphate (PIP₂), which binds to positively charged regions of the N-terminus of the β -subunit of ENaC, thereby maintaining the ENaC channel open and active. High dietary NaCl intake increases the luminal release of ATP and UTP, at least in part via a connexin30 (Cx30)-dependent mechanism in intercalated cells (IC). This activates apical P2Y₂ receptors and phospholipase C (PLC), which hydrolyzes and lowers the concentration of PIP₂. The resulting conformation change in the N-terminus of β -ENaC lowers ENaC P_O and activity, and increases Na⁺ excretion. See text for details. IP₃, inositol trisphosphate. Modified from ².

Figure 4. A proposed model for cell volume-dependent ATP release and water transport inhibition by P2Y₂ receptor activation in IMCD cells

Arginine-vasopressin (AVP) activates vasopressin V₂ receptor (V₂R) to stimulate aquaporin-2 mediated water entry which increases cell volume. The latter increases basolateral and apical release of ATP (and potentially UTP?) by unknown mechanism. P2Y₂ receptor activation inhibits water reabsorption via multiple signaling pathways and stimulates volume regulatory K⁺ channels as shown in other cell types. These effects help to stabilize cell volume. In response to an acute water load, the relative hypotonic extracellular fluid may increase cell volume. The resulting release of nucleotides and activation of P2Y₂ receptor stabilizes cell volume and accelerates the excretion of free water before circulating AVP levels fall. AC, adenylyl cyclase; AQP2, -3, -4, aquaporin-2, -3, -4; COX1, cyclooxygenase 1; CREB, cAMP responsive element-binding protein; DAG, diacylglycerol; EP₃, PGE₂ E₃ receptor; ET-1, endothelin 1; ETB, endothelin B receptor; G_i, inhibitory G protein; G_q, G_s, stimulatory G protein; IP₃, inositol trisphosphate; P, phosphorylation; PGE₂, prostaglandin E₂; PLC, phospholipase C; PKA, protein kinase A; PKC, protein kinase C; UT-A1, urea transporter A1. Modified from ².

Figure 5. Indirect evidence for a positive relationship between collecting duct cell volume and ATP release, and a role of P2Y₂ receptors in stabilizing cell volume

Quantitative urine collection was performed in P2Y₂^{-/-} mice and littermate wild types (WT) in metabolic cages over 2 hours following acute vasopressin V₂ receptor inhibition (V₂R-I) or acute oral water loading (WL). (A) V₂R-I and WL both increased urinary flow rate but induced opposite effects on urinary ATP excretion. Urinary flow rate is not a good predictor of urinary ATP excretion. (B) V₂R-I is expected to reduce CD water uptake and thus CD cell volume. In contrast, the reduction in extracellular tonicity associated with WL may increase CD cell volume. If an increase in cell volume induces ATP release, which stabilizes cell volume via inhibition of water uptake through a P2Y₂ receptor-mediated mechanism, then ATP release is expected to be enhanced in P2Y₂^{-/-} mice. This influence should be minimized by reducing water uptake by V₂R-I and maximized by water loading-induced cell swelling. Based on 23 and modified from ⁷³.

Table 1

Expression of P2Y receptors along the native tubule and collecting duct system

	proximal tubules	loop of Henle	collecting duct
P2Y ₁	IM: PST-A ¹⁵ ; mRNA: PCT ⁹⁰	mRNA: tDL, tAL, mTAL ⁹⁰	mRNA: OMCD ⁹⁰
P2Y ₂	mRNA: PCT ^{90;91} ; PST ⁹¹	IM: tAL, mTAL (intra), cTAL (intra) ¹⁵ ; mRNA: tDL, tAL ^{90;91} , mTAL ^{90;91} , cTAL ⁹¹ , mouse mTAL ³¹	IM: CCD (PC and IC, PC: intra) ⁴⁶ ; OMCD (PC and IC, PC: intra-A/B) ⁴⁶ ; MCD IC ¹⁵ ; IMCD (A>B) ⁹¹ ; IMCD (PC and IC, PC: B>A) ⁴⁶ ; WB: IMCD ⁹¹ ; mRNA: CD ⁴⁶ ; CCD ⁹¹ , OMCD ^{90;91} , IMCD ⁹¹
P2Y ₄	IM: PCT (B) ¹⁵ ; mRNA: PCT ⁹⁰	mRNA: tAL, mTAL ⁹⁰	IM: CCD, OMCD, IMCD (PC and IC, PC: all A); mRNA: OMCD ⁹⁰ ; CD (NaCl restriction) ⁴⁶
P2Y ₆	mRNA: PCT, PST ⁹²	mRNA: tDL, mTAL, cTAL ⁹² , mouse mTAL ³¹	IM: CCD, OMCD, IMCD (PC and IC, PC: all intra-A) ⁴⁶ ; mRNA: CD ⁴⁶ ; CCD, OMCD ⁹²

P2Y_{11–14} have not been identified unequivocally in the kidney and therefore were not included in the table. (A), apical; (B), basolateral; CCD, cortical collecting duct; CD, collecting duct; cTAL, cortical thick ascending limb; IC, intercalated cells; IM, immunostaining; IMCD, inner medullary collecting duct; (intra), intracellular; OMCD, outer medullary collecting duct; PC, principal cell; PCT, proximal convoluted tubule; PST, proximal straight tubule; tAL, thin ascending limb; mTAL, thick ascending limb; tDL, thin descending limb; WB, Western blotting. All studies performed in rat kidney unless otherwise stated. Modified from ¹.

Table 2

Effects of P2Y receptor activation in proximal tubules

Receptor	Effect	Method (site of effect) criteria for receptor subtype
P2Y	$[Ca^{2+}]_i$ ↑ via PLC, TRP channel	human-derived renal PT cell line (HKC-8) ⁹³
P2Y ₁ , P2Y ₂ -like	$[Ca^{2+}]_i$ ↑	isolated rabbit or rat PT (B) ^{80;90;94}
P2Y ₁	bicarbonate reabsorption ↓	rat micropuncture in vivo (A) ⁹⁵
P2Y ₂ -like	$[Ca^{2+}]_i$ ↑, gluconeogenesis ↑, Ca^{2+} and PLC-dependent	rat renal cortical tubule suspensions or isolated rat PT (B) ^{96;97}
P2Y ₆	$[Ca^{2+}]_i$ ↑ and IP ↑	isolated rat PT (B) ⁹²

(A), apical effect; (B), basolateral effect; IP, inositol phosphates; PLC, phospholipase C; TRP, transient receptor potential. Modified from ¹.