Potential role of purinergic signaling in urinary concentration in inner medulla: insights from P2Y2 receptor gene knockout mice

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The toxicity of the medullary interstitial fluid needed for osmotic water reabsorption by increasing the transport of NaCl in the outer medulla (5) and by facilitating the generation of urea concentration gradients in the deep inner medulla (2, 34, 35). Thus both an increase in the transepithelial water permeability in the medullary collecting duct and increased medullary interstitial toxicity are required for water reabsorption to maintain body water homeostasis and to concentrate urine.

After acute administration, AVP directly increases the transepithelial water flow in the medullary collecting duct by increasing the apical plasma membrane targeting of AQP2 protein in principal cells (27). Chronic infusion of AVP or its V2 receptor-specific analog 1-desamino-8-D-arginine vasopressin or desmopressin (dDAVP) increases the protein abundance of AQP2 by transcriptional activation of the AQP2 gene (28), as well as by increasing its apical membrane targeting in collecting duct principal cells (4). Both of these effects are mediated by the ability of AVP to induce an increase in intracellular cAMP levels (1, 13, 25, 29, 47).

AVP also increases the sodium-absorbing capability of the medullary thick ascending limb by stimulating the activity of the Na-K-2Cl cotransporter (5). The net effect is to increase medullary interstitial toxicity, which is required for rapid absorption of large amounts of water through adjacent collecting ducts in the outer medulla.

The major urea transporters, UT-A and UT-B, mediate facilitated diffusion of urea across tubular, interstitial, and vascular compartments and thus maintain the interstitial osmolar gradient necessary for the absorption of water through AQP2 in the deep inner medulla (1, 34). AVP increases UT-A2 expression by transcriptional regulation via a cAMP-responsive element. However, the increase in UT-A1 and UT-A3 expression following chronic AVP infusion appears to be due to an increase in medullary toxicity. The resulting increase in interstitial toxicity may activate UT-A promoter I through its toxicity enhancer element, leading to increased transcription of UT-A1 and UT-A3 (34).

In recent years, extracellular nucleotides (ATP or UTP), which act through purinergic P2 receptors, have emerged as potent regulators of renal transport functions (37, 38, 44). The P2Y2 receptor (P2Y2-R) is the most widely studied P2Y subtype of P2 receptors in the kidney. It is a G protein-coupled
extracellular nucleotide receptor that responds to both purine (ATP) and pyridine (UTP) nucleotides (38). Pharmacological, functional, and molecular approaches have localized the P2Y2-R to the medullary collecting duct (6, 14, 15). We documented that agonist stimulation of P2Y2-R in the medullary collecting duct results in (1) direct inhibition of osmotic water flow by a protein kinase C-dependent mechanism (14); and (2) production and release of PGE2 by a cyclooxygenase-1-dependent mechanism (46). PGE2 is a potent antagonist of AVP action on the medullary collecting duct (11, 26, 32, 49). Hence, the net effect of P2Y2-R activation is antagonistic to the actions of AVP on the medullary collecting duct.

The above observations prompted us to examine the extent of purinergic signaling in tonically counteracting the effect of AVP by studying P2Y2-R gene knockout mice (P2Y2-R KO). We determined the protein abundances of AQP2 and UT-A isoforms of purinergic signaling in tonically counteracting the effect of AVP on the medullary collecting duct (11, 26, 32, 49). Hence, the net effect of P2Y2-R activation is antagonistic to the actions of AVP on the medullary collecting duct.

MATERIALS AND METHODS

Experimental animals. All animal procedures were carried out according to the protocols approved by the Institutional Animal Care and Use Committees of the VA Salt Lake City Health Care System and the University of Utah. Animals were housed in the Veterinary Medical Unit (VMU) of the VA Salt Lake City Health Care System. This facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (International) and approved by the US Department of Agriculture and the Office of Laboratory Animal Welfare of the US Public Health Service.

Breeders of mice deficient in P2Y2-R (P2Y2-R KO) and the corresponding WT mice were kindly supplied by Dr. Beverly Koller (University of North Carolina at Chapel Hill, Chapel Hill, NC) (3, 12). According to Koller and her associates, P2Y2-R mice were generated by targeted mutagenesis of the P2Y2-R gene in mouse embryonic stem cells. Chimeric mice generated with P2Y2-R-targeted E142aTG cell lines were bred to B6D2 mice. RNA isolated from the kidney confirmed the complete loss of P2Y2-R in the P2Y2-R KO mice. To determine the protein abundance of P2Y2-R in the WT mice, we used the approach of feeding a gel diet for evaluation of ovalbumin expression in ovalbumin mice (9, 10). Groups of age- and sex-matched WT and KO mice under basal conditions. The gel diet approach is specific for the WT mice only. The other set of primers is targeted to the corresponding WT mice were kindly supplied by Dr. Beverly Koller (University of North Carolina at Chapel Hill, Chapel Hill, NC) (3, 12). According to Koller and her associates, P2Y2-R mice were generated by targeted mutagenesis of the P2Y2-R gene in mouse embryonic stem cells. Chimeric mice generated with P2Y2-R-targeted E142aTG cell lines were bred to B6D2 mice. RNA isolated from the kidney confirmed the complete loss of P2Y2-R in the P2Y2-R KO mice. No differences were detected between P2Y2-R KO and WT mice on histological examination of organs analyzed, including the kidney, heart, testis, pancreas, liver, trachea, lungs, salivary glands, and gastrointestinal tract (3, 12).

Mouse breeding colonies were established in the Veterinary Medical Unit of VA Salt Lake City Health Care System, and the bred mice were genotyped by performing PCR on DNA extracted from the tail clips. Two sets of primer pairs, as reported by Leipziger and associates (24), were used to differentiate the P2Y2-R KO and WT mice. One set of primers is targeted to the P2Y2-R gene and thus is specific for the WT mice only. The other set of primers is targeted to the neomycin cassette in the DNA construct used to disrupt the P2Y2-R gene and thus identifies the P2Y2-R KO mice. Mice used in the studies were between 2 and 5 mo of age.

Gel diet study. Since initial studies with ad libitum access to water and solid food did not reveal a consistent renal phenotype in P2Y2-R KO mice, we used the approach of feeding a gel diet for evaluation of the WT and KO mice under basal conditions. The gel diet approach is well characterized in our laboratories to evaluate the renal phenotype of other transgenic mice (9, 10). Groups of age- and sex-matched P2Y2-R KO and WT mice (n = 6/group) were fed a specially formulated gel diet as described before (9, 10). The gel diet, containing all the nutrients and normal content of water (70% by weight of the gel) and sodium (0.23%), was prepared by reconstituting a defined quantity of PMI Micro-Stabilizer Rodent Powdered Diet (LD101, Lab Diet, Richmond, IN) in water with added gelatin and then microwav-
optimal gray scale were digitized by photographic imaging with a high-pixel digital camera. Relative band densities were quantified using Un-Scan-It software (Silk Scientific, Orem, UT). Alternatively, after washing of the primary antibody, the PVDF membranes were incubated with Alexa Fluor 680-linked anti-rabbit IgG (Molecular Probes, Eugene, OR). The secondary antibody was visualized and quantified using infrared detection with the Odyssey protein analysis system (Liorc, Lincoln, NE). Densitometric results are computed as volume integrated values expressed as percentage of the mean values in control groups (100%). Equality of protein loading and/or transfer efficiency was checked by either staining the separated proteins in parallel gels by Coomassie blue (Gel Code Blue, Pierce Endogen) or staining the PVDF membranes with Ponceau S stain after immunoblotting. Transfer efficiency was also checked by monitoring the transfer of colored protein markers from gel to membrane, and in some cases by staining the gels with Coomassie blue after transfer. Digital images were used to determine the relative band densities.

Statistical analysis. Quantitative values are expressed as means ± SE. Differences between the means of two groups were analyzed by an unpaired t-test or Mann-Whitney nonparametric test. Differences among the means of more than two groups were analyzed by ANOVA followed by the assessment of differences by Tukey-Kramer’s multiple comparison test of Fisher’s least significant difference (protected t-test) to determine which groups are significantly different. P values <0.05 were considered significant.

RESULTS

Gel diet study. Stable basal conditions were achieved by feeding mice a gel diet containing a normal amount of water for 10 consecutive days. Figure 1 shows results obtained from these mice during the last 3 experimental days. As shown in Fig. 1, the mean water intake in the P2Y2-R KO mice was modestly low (~12%; P < 0.04). However, there was a prominent decrease in urine output (~32%; P < 0.02) associated with a prominent increase in urine osmolality (~38%; P < 0.003) in the P2Y2-R KO mice compared with the WT mice. Thus there were quantitative differences in water intake and urine output between the WT and KO mice. Due to these differences, the mean values of percent urinary excretion of water during the 3 experimental days between the WT and KO mice were also significantly different (35.4 ± 1.8% in WT vs. 27.3 ± 3.3% in KO, P < 0.05). The observed differences in the percent water excretion through urine manifested over the period of time that the mice were kept on a gel diet and were not seen in the beginning of the study (data not shown). The total osmolar excretion, however, did not differ significantly between these two groups (P = 0.35). Terminal serum osmolalities in the WT and P2Y2-R KO mice were essentially similar (299 ± 8 vs. 297 ± 6 mosmol/kgH2O, P = 0.73) (see Fig. 2A), indicating that these mice were not in gross water imbalance.

The apparent increase in urinary concentrating ability of the P2Y2-R KO mice was associated with a 1.8-fold higher (P < 0.006) protein abundance of the AVP-regulated AQP2 water channel in the whole medulla (Fig. 3). Interestingly, there were no significant differences in the terminal serum AVP levels (Fig. 2B) between these two groups (WT: 11.3 ± 0.7 pg/ml, n = 4 vs. P2Y2-R KO: 10.7 ± 0.5 pg/ml, n = 5; P = 0.678), proving that the observed significant increases in the urinary concentrating ability and protein abundances of AQP2 were not due to higher circulating AVP levels. Furthermore, the mean values of the PGE2 metabolite in the urine of these two groups of mice also did not differ from each other when expressed either as picograms per day or nanograms per milligram creatinine (data not shown).

We further examined the whole medullary tissue samples from these mice for the protein abundances of UT-A1 (expressed in inner medullary collecting duct) and UT-A2 (expressed in thin descending thin limb of Henle). Figure 4 shows the abundances of UT-A1 and UT-A2 proteins in the whole medullary tissue samples (outer medulla+inner medulla) of WT and KO mice under basal conditions. The KO mice have significantly higher (166 ± 13%, P < 0.02) UT-A1 protein abundance compared with the WT mice. The KO mice also showed significantly higher protein abundance of UT-A2 (129 ± 5%, P < 0.05) compared with the WT mice.

Chronic dDAVP administration study. Chronic administration of dDAVP, a vasopressin V2 receptor-specific analog, is known to exert its effect mostly on the collecting duct. When chronically infused at a rate of 1 ng/h for 5 days, dDAVP significantly increased urine concentration in both in WT and KO mice (Fig. 5). The day 0 values shown in Fig. 5 correspond to the urine samples collected just before implantation of the minipumps. As such, the values shown for both day 0 and day 1 are baseline values. The urine osmolality in WT and KO mice increased steadily and reached a plateau by day 2, at which point the mean urine osmolality in KO mice was
significantly higher than the mean urine osmolality in WT mice (WT: 2,723 ± 186 mosmol/kgH2O vs. KO: 3,093 ± 281 mosmol/kgH2O, n = 5, P < 0.05).

When inner medullary tissue samples were analyzed by Western blotting, the AQP2 protein abundance in the KO mice was significantly higher (214 ± 14%, P < 0.01) compared with the WT mice (Fig. 6). Although not statistically significant, this 2.14-fold higher protein abundance seen in KO mice after chronic dDAVP infusion corresponds to 19% further increase compared with the protein abundance seen in the same group under stable basal conditions (1.8-fold; Fig. 3).

Since chronic dDAVP is known to increase the expression of AQP3, the basolateral water channel in the collecting duct, we also examined its protein abundance in the chronically dDAVP-infused WT and KO mice. There was a significant 2.6-fold higher protein abundance of AQP3 in KO mice compared with the WT mice (Fig. 7). We further analyzed the inner medullary samples for the expression of UT-A1 protein (UT-A2 protein cannot be detected in inner medulla). Figure 8 shows that KO mice have significantly higher protein abundance of UT-A1 protein (222 ± 19%, P < 0.01) in the inner medulla compared with the WT mice. Thus chronic dDAVP infusion resulted in 2.14-, 2.6-, and 2.22-fold higher protein abundances of AQP2, AQP3, and UT-A1, respectively, in the inner medulla of KO mice compared with WT mice.

Acute AVP treatment. We also examined the acute effect of AVP on the protein abundance of UT-A1 in KO and WT mice. Acute treatment of WT mice with AVP caused a significant increase (139 ± 9%) in the protein abundance of UT-A1 compared with the untreated control group (Fig. 9). However, the corresponding increase in KO mice is slightly higher (154 ± 6%; Fig. 9). We did not see consistent changes in AQP2 protein levels in response to acute vasopressin treatment (data not shown).

DISCUSSION

In this communication we examined the potential role of P2Y2-R-driven purinergic signaling on the urinary concentrating mechanism in the renal medulla by subjecting P2Y2-R gene KO and WT mice to stable basal conditions, or to acute or chronic vasopressin administration. Under stable basal conditions achieved by feeding the animals a gel diet, we documented that genetic deletion of P2Y2-R results in a significant increase in urinary concentrating ability associated with a marked increase in the protein abundance of the AVP-regulated apical water channel, AQP2, and UT-A1 in the medulla, despite normal circulating AVP levels. We also documented that following chronic dDAVP infusion into the KO mice, these significant differences not only persisted but showed numerically higher values. Second, we showed that following chronic dDAVP infusion, KO mice had a much higher protein abundance of AQP3, the basolateral collecting duct water channel. Third, we showed that while both WT and KO mice respond to acute AVP treatment with increased UT-A1 protein in the inner medulla, the increase in KO mice is numerically higher. Thus these findings suggest that purinergic signaling plays an overarching role in the urinary concentration mechanism by tonically regulating the protein abundances of key membrane transporters and channels. Conversely, our study also demonstrates that in the absence of the P2Y2-R, the medullary collecting duct is sensitized to the action of AVP.

The collecting duct system accounts for the regulated absorption of ~15% of filtered water, which determines the volume and concentration of the final voided urine (28, 29).
AVP, acting through its V2 receptor, a G protein-coupled receptor, in collecting duct principal cells, activates the membrane-bound adenylyl cyclase to produce cAMP as a second messenger. The short- and long-term cellular effects of AVP, such as apical membrane targeting of AQP2 protein, and transcriptional activation of the AQP2 gene, respectively, are mediated by the sustained elevation of cellular cAMP levels (1, 13, 25, 29, 47). However, a variety of agents, such as endothelin, PGE2, extracellular nucleotides (ATP/UTP), adenosine, β-adrenergic agonists, atrial natriuretic peptide, and others are known to modulate the function of the collecting duct system. Some of these locally produced autocrine and/or paracrine factors, such as endothelin, PGE2, and extracellular nucleotides, are known to oppose the action of AVP and decrease the water-absorbing capacity of the collecting duct system (8, 11, 14, 22, 26, 32, 33, 49). The role of these autocrine or paracrine agents in modulating collecting duct water transport in healthy and disease conditions, especially in pathophysiological conditions associated with AVP resistance, is not well defined. This is mostly because of the nature of their production and rapid degradation locally in the kidney, which limits the tools available for investigation of their functional roles.

Despite these limitations, we have been investigating the role of the purinergic system in the medullary collecting duct function under physiological and pathophysiological conditions. We identified that agonist (ATP/UTP) activation of P2Y2-R in the medullary collecting duct results in direct inhibition of AVP-induced water flow (14) and also increases the production of PGE2 (46). As stated earlier, PGE2 is a known antagonist of AVP action on the collecting duct (26, 32, 49). Thus, in agreement with our previous findings, the observed significant increase in urinary concentrating ability and AQP2 protein abundance in the presence of normal serum AVP levels under stable basal conditions in P2Y2-R KO mice suggests that medullary collecting ducts of these mice are more...
sensitive to circulating AVP levels. These findings are also consistent with the general concept that in the collecting duct, the cAMP system mediated by AVP, the phosphoinositide-signaling pathway mediated by P2Y2-R, and other receptors are mutually opposing (38, 43). So, genetic deletion of P2Y2-R apparently tilted the balance of signaling pathways in favor of a cAMP-mediated system. Furthermore, since the percent excretion of water through urine was significantly lower in KO mice vs. WT mice in our gel diet-fed series, it is possible that other routes of water excretion, such as feces, in KO mice may be more active.

During the course of this study, Vallon and associates (30) reported that under basal conditions (ad libitum access to water and solid food) the P2Y2-R KO mice had greater medullary expression of AQP2, and AVP-dependent renal cAMP formation, and water reabsorption despite similar urinary AVP levels compared with WT mice. Our study extends the earlier report by documenting comparable circulating AVP levels in P2Y2-R KO and WT mice under stable basal conditions. In addition, consistent with the findings of Vallon and associates, under basal conditions with free access by the animals to food and water, we also did not see significant differences in urine output or osmolality between the P2Y2 receptor KO and WT mice.

AVP also increases the protein abundance and/or activity of urea transporters, which in turn increases the urea concentration gradients in the deep medulla needed for the concentration of urine (2, 34, 35). This prompted us to examine the protein abundance of UT-A isoforms in the medulla of P2Y2-R KO and WT mice. Immuneblots using whole medulla (outer + inner) of mice under basal conditions showed 1.66-fold higher protein abundance of the inner medullary collecting duct urea transporter UT-A1 in KO mice compared with WT mice. These blots of the whole medulla also revealed 1.29-fold higher protein abundance of UT-A2, the urea transporter of the thin descending limb of the loop of Henle, in KO mice compared with WT mice. Thus concomitant increases in the protein abundances of these two urea transporters should enable KO mice to generate significantly higher urea concentration gradients in the medulla compared with WT mice. The potential increase in urea concentration gradients in the deep medulla that one can expect under these conditions, associated with a significant increase in AQP2 protein abundance seen in KO mice, apparently enabled these mice to concentrate urine to a higher degree compared with WT mice.

Since our interest is primarily in the role of purinergic signaling in the regulation of transport functions of the inner medullary collecting duct, in this study we further focused on the effect of chronic or acute administration of dDAVP or AVP on the protein abundance of UT-A1 expressed in the inner medullary collecting duct. UT-A2 protein abundance is very low in the inner medulla, as it is expressed in the descending...
thin limb of Henle’s loop (2, 34, 35). Chronic dDAVP infusion, which specifically stimulates the vasopressin V2 receptor present in the collecting duct principal cells, resulted in relatively higher urinary osmolality levels in KO mice compared with WT mice, although the difference between these two groups was found to be statistically significant only on day 2 of dDAVP infusion. After 5 days of dDAVP infusion, AQP2 protein abundance in the inner medulla of KO mice was 2.22-fold higher compared with that in WT mice. This difference is more pronounced compared with the difference seen under basal conditions in KO and WT mice (1.8-fold; Fig. 3).

We also found that there is a marked increase (2.6-fold) in the protein abundance of AQP3, the basolateral water channel of the collecting duct. AQP3 protein is known to increase following chronic dDAVP infusion (28), although it is not affected by an acute administration of AVP. In parallel, we found that UT-A1 protein abundance in dDAVP-infused KO mice is 2.14-fold higher compared with the dDAVP-infused WT mice. This difference is also numerically higher than that observed between KO and WT mice under basal conditions (1.66-fold). Thus it appears that chronic dDAVP infusion has a more profound effect on P2Y2-R KO mice than on WT mice. This

![UT-A1 Protein in Inner Medulla](image1)

**Fig. 8.** UT-A1 protein abundance in the inner medulla of P2Y2-R KO and WT mice chronically infused with dDAVP. Groups of WT and KO mice (n = 5/group) were infused with dDAVP at the rate of 1 ng/h for 5 days via subcutaneous osmotic minipumps and were euthanized on day 5. Inner medullary tissue samples were processed by semiquantitative immunoblotting for UT-A1 protein. *Left:* representative blots probed with an antibody specific for COOH terminus of UT-A protein, where each lane corresponds to a sample from 1 mouse. The intensity of UT-A1 bands (97–117 kDa) was greater in the KO compared with WT mice. *Right:* densitometry of UT-A1 bands in WT and KO mice. Bars represent means ± SE, expressed as the percentage of mean values in WT mice (n = 5). *P < 0.01 vs. WT mice.

![UT-A1 Protein in Inner Medulla](image2)

**Fig. 9.** Effect of acute administration of AVP on the protein abundance of UT-A1 in the inner medullas of P2Y2-R KO and WT mice. Groups of WT and KO mice were either subcutaneously injected with AVP (0.2 unit/mouse) or none (–AVP). All AVP-injected mice were euthanized exactly 45 min after injection. Inner medullary tissue samples were processed by semiquantitative immunoblotting. *Top:* representative blots probed with an antibody specific for COOH terminus of UT-A protein, where each lane corresponds to a sample from 1 mouse. In both WT and KO mice the intensity of UT-A1 bands (97–117 kDa) was greater after acute treatment with AVP. *Bottom:* densitometry of UT-A1 bands WT and KO mice. Bars represent means ± SE, expressed as the percentage of mean values in the corresponding –AVP group (n = 5). *P < 0.05 or better vs. corresponding –AVP group.
may be due to the lack of tonic inhibitory effect of purinergic signaling on AVP in P2Y2-R KO mice.

It is interesting to note that the markedly higher protein abundances of AQP2 in the inner medullas of chronically dDAVP-infused KO mice vs. WT mice were not accompanied by a similar degree of difference in urine osmolalities. Thus it appears that there may be some sort of dissociation in the expected linear relationship between AQP2 protein abundance in the medulla and urinary concentration. This notion derives support from the study published by the Vallon group (30), where they documented an interesting phenomenon. They showed that under basal or unchallenged conditions, despite the lack of significant differences in urinary parameters, there was a twofold higher protein abundance of AQP2 in the renal medulla of P2Y2-R KO mice vs. WT mice. They also documented that rapid movement to the plasma membrane (19), an increase in phosphorylation (50), and an increase in activity (45). AQP2 is also known to move to the plasma membrane in response to acute AVP, resulting in increased water channel activity (27). However, the acute effect of AVP on the protein abundance of UT-A1 is not known. So, we examined the acute effect of AVP on the protein abundance of UT-A1 in the inner medulla of KO and WT mice. UT-A1 responds to acute stimulation by AVP with rapid movement to the plasma membrane (19), an increase in phosphorylation (50), and an increase in activity (45). AQP2 is also known to move to the plasma membrane in response to acute AVP, resulting in increased water channel activity (27). However, the acute effect of AVP on the protein abundance of UT-A1 is not known. So, we examined the acute effect of AVP on the protein abundance of UT-A1 in the inner medulla of KO and WT mice. In response to acute AVP stimulation, UT-A1 protein abundance was significantly increased in both WT (139 ± 9%) and KO (154 ± 6%) mice. However, the increases in these two groups were not significantly different from each other. In this respect, the acute and chronic effects of AVP/dDAVP on UT-A1 protein abundance in WT and KO mice were very different. It is possible that higher doses of AVP or longer durations of stimulation may unmask potential differences between these two groups of mice. Pending such studies, at this stage it appears that at least under the standard or conventional methods of studying the acute AVP effect, there were no significant differences between the WT and KO mice with respect to UT-A1 protein abundance in the inner medulla. Thus in this respect the acute effect of AVP is different from the chronic effect of dDAVP. We could not detect consistent changes in AQP2 protein abundance in response to acute AVP stimulation. This is not surprising, given that the acute effect of AVP on AQP2 is mostly confined to the redistribution of AQP2 protein within the cell, i.e., membrane targeting from a subapical pool. We also examined the effect of prior water loading on the acute effect of AVP on UT-A1 in WT and KO mice. The results were not consistent with a considerable variation even within each group (data not shown here).

Thus our studies reveal an overarching role for purinergic signaling in the urinary concentration mechanism by tonically inhibiting the stimulating effect of AVP. To be effective, as a local autocrine or paracrine regulator of AVP action, purinergic signaling should be finely modulated by rapid release of ATP and related nucleotides into extracellular milieu followed by rapid hydrolysis by the ecto-nucleotidases. There is ample evidence in the literature documenting that almost all cells in the body are capable of releasing ATP and related nucleotides by a process of either regulated exocytosis or through specific membrane transport mechanisms, such as anionic channels (23, 37, 38), as well as a variety of ecto-nucleotidases, such as nucleoside triphosphate diphosphohydrolases (NDTPases) and nucleotide pyrophosphatases (NPPs) that can rapidly hydrolyze the extracellular nucleotides (31, 39, 48). We showed that medullary collecting ducts of the rat are capable of releasing substantial amounts of ATP (38), and we localized NTPDase1 in ascending thin limbs of Henle’s loop, which are in close apposition to the collecting ducts (16). Future studies should address the integrative perspectives of the interactions of vasopressin, purinergic and prostanoid systems, or signaling in the renal medulla as they relate to the urinary concentration mechanism. Published data from our laboratories already point to the existence of such an integrative mechanism in the renal medulla (18, 41, 42, 46).

We also believe that purinergic signaling may play an important role in pathophysiological conditions, especially conditions associated with vasopressin resistance. It is interesting to note here that we previously showed that UT-A1 protein abundance was reduced to 50% in the inner medullary tip and to 25% in the inner medullary base of lithium-fed rats compared with a control group of rats (20). Lithium is known to induce vasopressin-resistant polyuria (36). Recently we obtained evidence that purinergic signaling is markedly up-regulated in the inner medullas of lithium-fed rats (42). These two studies, taken together with the current study on P2Y2-R KO mice, suggest that the observed reduction in UT-A1 protein in the medulla of lithium-fed rats may be due in part to the increased activity of purinergic signaling. Future studies should establish the extent of the contribution of purinergic signaling in the pathophysiological conditions associated with dysregulation of UT-A1 in the inner medulla.

Finally, we must acknowledge the inherent limitations of our mouse model. As with any global KO mouse model, the observed changes in whole-animal physiology may also reflect the effects of global P2Y2-R deletion, such as changes in the brain (neurohormonal?) or absorption in the gut. Only a model of collecting duct-specific deletion of P2Y2-R can eliminate such possibilities. Pending the generations of such cell-specific gene KO mice, at this stage this is the best available model for investigating the role of P2Y2-R in urinary concentration.

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