P2Y2 receptor-mediated release of prostaglandin E2 by IMCD is altered in hydrated and dehydrated rats: relevance to AVP-independent regulation of IMCD function

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cyclooxygenases; extracellular nucleotides; arginine vasopressin; purinergic; aquaporin; inner medullary collecting duct; prostaglandin E2

THE REABSORPTION OF WATER by the kidney is crucial for the conservation of body water and excretion of concentrated urine. Within the kidney, the collecting duct system is the site of regulated water reabsorption. It is under the control of the neurohypophyseal hormone arginine vasopressin (AVP). AVP, acting through its V2 receptor, a G protein-coupled receptor on the collecting duct principal cells, activates membrane-bound adenyl cyclase to produce cAMP as a second messenger.

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cAMP levels in a protein kinase C-dependent manner (16). Recently, we showed that P2Y2 receptor stimulation triggers the production and release of PGE2 by rat IMCD preparations in a cyclooxygenase-1 (COX-1) activity-dependent manner (36). Thus, our studies indicate that in rat IMCD, signaling through P2Y2 receptor drives the production of PGE2. This has important physiological significance, because PGE2 is known to affect the transport of water, salt, and urea in IMCD (25, 28, 29).

More recently, we observed that the expression of P2Y2 receptor mRNA and protein in the inner medulla is altered by the hydration status of the animals. Thus, in hydrated rats the expression of P2Y2 receptor mRNA and protein in the inner medulla is significantly higher compared with the dehydrated rats (18). This prompted us to hypothesize that the altered expression of P2Y2 receptor in hydrated and dehydrated conditions may be associated with changes in P2Y2 receptor-driven PGE2 production by the IMCD. In this study, we examined that possibility.

**MATERIALS AND METHODS**

**Experimental animals.** The animal experiments were carried out according to the protocols approved by the Institutional Animal Care and Use Committees of the University of Utah and VA Salt Lake City Health Care System. Specific pathogen-free male Sprague-Dawley rats (Harlan, Indianapolis, IN) were housed two or three per cage in the Veterinary Medical Unit of the VA Salt Lake City Health Care System. This is an American Association for Accreditation of Laboratory Animal Care (AAALAC)-accredited and US Department of Agriculture (USDA)- and Public Health Service (PHS)-approved animal facility. The rats were maintained in pathogen-free state and fed ad libitum a commercial rodent diet. They had free access to drinking water unless restricted by the experimental protocol. The rats were acclimatized to the housing conditions for about a week before experiments were conducted. The rats weighed 294 ± 9 g (means ± SE) at the time of euthanasia.

**Hydration and dehydration of rats.** Hydration and dehydration of rats were achieved according to the protocol described earlier (18). Briefly, hydrated rats received 600 mM sucrose (commercially available dietary cane sugar) in tap water for 2 days (48 h) as the sole drinking fluid. The sweetness of the sucrose solution caused the rats to drink large amounts of fluid and thus established a hydrated state associated with diuresis. The dehydrated rats were deprived of water for 2 days (48 h). In addition to the hydrated and dehydrated rats, a group of normal rats was included, which received only tap water ad libitum. All rats had free access to pelleted chow.

**Collection and analysis of urine samples.** Urine samples were collected from rats by placing them in individual plastic metabolic cages during the last 24 h of the experimental period, just before euthanasia. After collection, volumes of 24-h urine samples were noted. Aliquots of urine samples were centrifuged to remove suspended particles. The supernatants were used to measure osmolality of the urine samples to a single, stable derivative that could be easily quantified by EIA. According to the manufacturer’s instructions, briefly, to aliquots (500 μl) of each urine sample 150 μl of 1 M carbonate buffer were added and incubated at 37°C overnight. Then, 200 μl of phosphate buffer followed by 150 μl of EIA buffer (both supplied) were added. Urine samples were diluted 10-, 20-, or 50-fold. Fifty microliters of each dilution were used in the EIA. The absorbance of the final color product was read spectrophotometrically at 405 nm in a Spectra Max 250 microplate reader (Molecular Devices, Menlo Park, CA). The raw data from the plate reader were stored in a computer and analyzed using Soft MaxPro software (Molecular Devices). The measured amounts of PGE2 metabolite in the urine samples were normalized to the 24-h urine output and expressed as picograms of PGE2 metabolite per 24 h.

**Fractionation of preparations enriched in IMCD.** Fractions enriched in IMCD were prepared from rat kidney inner medullas as described previously (19, 36). Briefly, rats were euthanized by pentobarbital sodium overdose. Both kidneys were removed rapidly and chilled on ice-cold phosphate-buffered saline. The inner medullas or papillae were dissected. The dissected papillae were transferred to an isotonic HEPES-buffered physiological solution of the following composition (in mM): 135 NaCl, 0.5 KCl, 0.1 NaHPO4, 0.3 sodium acetate, 0.12 Na2SO4, 2.5 CaCl2, 1.2 MgSO4, 5 HEPES, and 5.5 d-glucose (pH 7.4, 300–310 mosmol/kgH2O). This solution was oxygenated by bubbling in 95% O2-5% CO2. Because earlier studies showed that PGE2 synthesis in IMCD is sensitive to increasing osmolality of the medium (15), we carried out all our experiments in ~300 mosmol/kgH2O medium to exclude variability due to osmolality. Depending on the nature of the experiment, renal papillae from three or four rats were pooled to obtain a sufficient amount of IMCD preparations for incubations. The pooled papillae were minced with a razor blade. The minced tissue was then digested at 37°C with collagenase B (3 mg/ml) and hyaluronidase (600 U/ml) in the same HEPES-buffered physiological solution for ~40–50 min with continuous oxygenation. Midway through the digestion process, DNase I (Sigma, St. Louis, MO) was added to the digestion mixture to digest stray DNA released from broken cells. The digestion mixture was intermittently aspirated into and pushed through a glass Pasteur pipette with a long, narrow tip to disperse the tubules into a uniform suspension. After digestion of the tubules into a uniform suspension, the IMCD fraction was separated from the non-IMCD elements (thin limbs and vasculature) by sedimentation by low-speed centrifugation and repeated washings. The final pellet was suspended in the oxygenated HEPES-buffered physiological solution to a protein concentration of ~1 mg/ml. The final suspension was kept on ice for ~30 min to allow for recovery of IMCD cells from stress. This preparation consisted of mostly IMCD segments or sheets of IMCD cells and a very few other non-IMCD elements. Characterization of IMCD preparations obtained by this procedure for enrichment in IMCD and for cell viability has been documented earlier (19, 36).

**Incubation of IMCD preparations.** Fractions enriched in IMCD were incubated with or without the addition of inhibitors of COX and/or ATPyS. At the end of the incubation, the amount of PGE2 released from the cells was assayed as described previously (36). Briefly, after keeping the IMCD preparations on ice for 30 min for recovery from stress, they were aliquoted into 1.5-ml plastic microtubes kept on ice. ATPyS stock solution (×10 final incubation concentration) was prepared in the same oxygenated HEPES-buffered physiological solution used for the preparation of IMCD suspensions. Stock solutions of valeroyl salicylate and NS-398 were prepared by dissolving in DMSO and subsequently used in the incubation. The aliquots of IMCD suspensions were warmed to 37°C for 5 min on heat blocks before the agents were added. When ATPyS alone was used without any COX inhibitors, the incubations were started immediately after the 5-min warm-up period and lasted for 10 or 20 min depending on the nature of the experiment.
When the COX inhibitors were used, the IMCD were initially warmed-up for 5 min. Then, they were preincubated with the inhibitors for 5 or 15 min depending on the type of the inhibitor used. This was followed by incubation for another 20 min after the addition of ATPyS. To control incubations that did not contain any inhibitor and/or ATPyS, equal volumes of vehicle (incubation buffer) were added, so that all the incubations had a final volume of 200 µl. Incubations were carried out either in triplicate or quadruplicate. To stop the reactions, chilled HEPES-buffered physiological solution (200 µl) was added and the tubes were kept on ice for a few minutes. The tubes were then centrifuged at 10,000 g for 10 min in a cold room (4°C). And 350 µl of the supernatant from each tube were transferred to a fresh tube and stored at −80°C until assayed for the PGE2 content. The pellets with the remaining 50 µl of incubation buffer were frozen at −20°C for protein assay.

**Assay of PGE2.** PGE2 content in the supernatants from the incubations was determined according to the instructions of the manufacturer using the Prostaglandin E2 EIA Kit Monoclonal (Catalog no. 514010; Cayman Chemical). The absorbance of the product was read spectrophotometrically at 405 nm in a Spectra Max 250 microplate reader (Molecular Devices). According to the manufacturer, this assay system has a specificity of 100% to PGE2 and PGE2 ethanolamide, 37.4% specificity to 8-iso PGE2, and 43 and 18.7% to PGE3 and PGE1. For the assays, frozen supernatants were thawed on ice and diluted with EIA buffer to yield final dilutions in the range of 1:200 to 1:2,000 with respect to the original incubation. The assays were run on 50 µl of these diluted samples. The raw data from the plate reader were stored in a computer and analyzed using Soft MaxPro software (Molecular Devices). The protein content in each sample was measured to normalize for cell population present in each sample. Protein pellets prepared from the IMCD cell suspension samples were thawed at room temperature. Cellular proteins were precipitated and delipidated by addition of methanol. After separation of methanol by centrifugation and drying, the protein pellets were dissolved in 0.05 N NaOH. Aliquots of the clear solutions thus obtained were assayed for the protein content by Coomassie Plus Protein Assay Reagent Kit (Pierce Endogen, Rockford, IL) according to the manufacturer’s instructions. The concentrations of PGE2 in the incubations were normalized with the corresponding protein content and expressed as nanograms of PGE2 released per milligram of protein. The day-to-day coefficient of variation of this assay in our hands was 3–13% in the linear range of the standard curve (10–90% binding of tracer).

**Agents.** ATPyS (−96% purity) was purchased from Calbiochem-Novabiochem (La Jolla, CA). 2-[[1-Oxypentyl]oxy]-benzoic acid (valeroyl salicylate; 99% purity) and N-[2-(cyclohexyloxy)-4-nitrophenyl] methanesulfonamide (NS-398; 99% purity) were purchased from the Cayman Chemical. DMSO (99.9% purity) and DNase I were from Sigma. Collagenase B was purchased from Roche Molecular Biochemicals (Indianapolis, IN), and bovine testes hyaluronidase was from Worthington Biochemical (Lakewood, NJ). All other chemicals used were of the highest purity available.

**Statistical analysis.** Values are expressed as means ± SE. Data were analyzed by one-way as well as by repeated-measures ANOVA followed by assessment of differences between the mean of the groups by Tukey-Kramer’s or Bonferroni’s multiple comparison tests. Unpaired t-test was used to directly compare two groups. P < 0.05 was considered significant. The statistical analyses were performed on a PC using GraphPad InStat Version 3.0 software package (GraphPad Software, San Diego, CA).

**RESULTS**

**Urine parameters in normal, dehydrated, and hydrated rats.** The urine outputs (Fig. 1A) and urine osmolarities (Fig. 1B) are shown for normal, dehydrated, and hydrated rats collected during the final 24 h of the experimental period. As shown in the Fig. 1, the dehydrated rats had low output of concentrated urine, whereas the hydrated rats had high output of dilute urine compared with the normal rats. Thus there are dramatic differences between dehydrated and hydrated groups with respect to urine volume and osmolality.

**Urinary PGE2 metabolite in normal, dehydrated, and hydrated rats.** Urine samples collected from normal, dehydrated, and hydrated rats during the last 24 h of the experimental period were assayed for PGE2 metabolite content after appropriate dilution, using a commercial EIA kit. The urinary excretion of PGE2 metabolite over 24 h in normal, dehydrated, and hydrated rats is shown in Fig. 2. In contrast to normal rats, the mean urinary excretion of PGE2 metabolite in dehydrated rats was only ~12%, whereas it was increased to ~142% in hydrated rats. Thus the hydrated group showed ~12-fold higher urinary excretion of PGE2 metabolite than the dehydrated group.

**P2Y2 receptor-stimulated release of PGE2 by the IMCD preparations from normal, dehydrated, and hydrated rats.** Fractions enriched in IMCD were prepared from inner medullas of normal, dehydrated, and hydrated rats and challenged with ATPyS, a nonhydrolyzable agonist of P2Y2 receptor. Figure 3 shows the amount of PGE2 released following agonist stimulation vs. unstimulated IMCD (vehicle control) in different groups of rats. Agonist stimulation of P2Y2 receptor in IMCD of normal rats caused a modest but significant 50% increase in PGE2 released when directly compared with the corresponding vehicle-treated group by unpaired t-test. In the hydrated group, P2Y2 receptor stimulation caused 136% increase in PGE2 release over the unstimulated values. In contrast, the dehydrated group did not show any change. Thus, when stimulated by ATPyS, IMCD from hydrated rats released >5- and 2.4-fold higher amounts of PGE2 compared with the dehydrated and normal groups, respectively. Basal amounts of PGE2 released during the experimental period by the unstimulated IMCD were numerically higher in hydrated and lower in dehydrated groups compared with the normal group, although these differences were not statistically significant when analyzed by ANOVA.
Time course of ATPγS-stimulated PGE2 release by IMCD from dehydrated and hydrated rats. The large differences in urine volume, osmolality, and urinary PGE2 metabolite observed in dehydrated and hydrated groups represented the cumulative changes that occurred over a 48-h period. Therefore, to assess the potential kinetic differences in the response between dehydrated and hydrated rats, we directly compared the time course of ATPγS-stimulated PGE2 release by IMCD prepared from these two groups. Figure 4 shows the time course of PGE2 release by IMCD from dehydrated and hydrated rats after stimulation with 50 μM ATPγS or no stimulation (vehicle). As shown in the figure, stimulated or unstimulated IMCD from dehydrated rats showed virtually no increase in the PGE2 release over time. On the other hand, unstimulated IMCD from hydrated rats showed a gradual increase over time, attaining statistical significance at 80 min. However, the stimulated IMCD from hydrated rats showed dramatic 3.3-, 5.2-, and 6.3-fold increases at 20, 40, and 80 min. Thus the IMCD from dehydrated and hydrated rats differed markedly in their ability to respond to ATPγS receptor-stimulated PGE2 release over time. These results were qualitatively similar to what we observed previously with IMCD from normal rats (36) but were quantitatively higher.

Effect of different concentrations of ATPγS on PGE2 release by IMCD from dehydrated and hydrated rats. In parallel to the time course of ATPγS-stimulated PGE2 release by IMCD, we tested ATPγS concentration response using IMCD from dehydrated and hydrated rats. Previously, we characterized the ATPγS concentration response in IMCD of normal rats (36). Figure 5 shows ATPγS concentration-response curve for the release of PGE2 by IMCD from dehydrated and hydrated rats following 10-min incubation. As shown in Fig. 5, in hydrated rats, there was an initial rapid increase even at 10 μM, followed by a more sustained increase up to 100 μM ATPγS concentration. In contrast, IMCD from dehydrated rats did not show significant increase of PGE2 release at any ATPγS concentration tested.

Effect of COX-1 inhibition on ATPγS-stimulated PGE2 release by IMCD from hydrated rats. Because IMCD of dehydrated rats showed virtually none or insignificant response to ATPγS-stimulated PGE2 release, here we examined the IMCD from the hydrated rats only with respect to their sensitivity to COX-1 inhibition. Figure 6 shows the effect of valeroyl salicylate, a selective, irreversible inhibitor or COX-1 (3), on ATPγS (50 μM)-stimulated PGE2 release by IMCD from hydrated rats. As shown in the figure, lower concentrations of valeroyl salicylate (30 μM) produced little inhibition of the ATPγS-stimulated PGE2 release. However, 300 μM valeroyl salicylate completely inhibited the ATPγS-stimulated release of PGE2 by the IMCD from hydrated rats. Although there were some differences in the level of PGE2 released during the

Fig. 2. Urinary excretion of PGE2 metabolite in normal, dehydrated, and hydrated rats. Twenty-four-hour urine samples were collected and processed for the assay of PGE2 metabolite as described in MATERIALS AND METHODS. Based on the volume of urine collected, urinary PGE2 metabolite excreted over 24-h period was calculated. Data show means ± SE for 6 rats in each group.

*Significantly different from the other 2 groups, P < 0.001 by ANOVA.

**Significantly different from the normal group, P < 0.001 by ANOVA.

Fig. 3. ATPγS-stimulated PGE2 release by inner medullary collecting duct (IMCD) preparations from normal, dehydrated, and hydrated rats. Frac-
tions enriched in IMCD were freshly prepared from pooled inner medullas in each group. Aliquots of IMCD fractions, after warming up to 37 °C for 5 min, were incubated in the presence of incubation buffer alone (vehicle) or 50 μM ATPγS for 20 min. Reactions were stopped by adding chilled incubation buffer. After the cellular elements were pelleted by centrifugation, the PGE2 concentrations in the supernatants were assayed by EIA and normalized to the protein contents of the incubations. Data shown are pooled from 2 experiments with a total of 6 rats per group. Incubations in each experiment were done in triplicate or quadruplicate. A: data as ng of PGE2 released per mg of cellular protein. B: same data as percentage of vehicle group in normal rats. *Significantly different from the corresponding vehicle group, P < 0.001 by ANOVA.

**Significantly different from the corresponding vehicle group, P < 0.002 by unpaired t-test.
experimental period between the vehicle controls and inhibitor alone incubations, these differences were not statistically significant. Thus P2Y2 receptor-stimulated PGE2 release by IMCD from dehydrated rats, similar to the IMCD of normal rats (36), was dependent on the activity of COX-1.

**DISCUSSION**

In this communication, we documented four major findings. First, we demonstrated that urinary excretion of PGE2, which reflects tubular fluid synthesis in vivo, is significantly higher in hydrated rats and lower in dehydrated rats compared with the normal rats. Second, we showed that ex vivo purinergic-stimulated PGE2 release by IMCD is markedly elevated in hydrated rats compared with the normal or dehydrated rats. Third, we demonstrated that purinergic-stimulated increase in PGE2 release by IMCD of hydrated rats is time and agonist concentration dependent. Finally, we document that purinergic-stimulated PGE2 release by IMCD of hydrated rats is mediated by COX-1 but not COX-2. Because PGE2 is known to affect transport of water, salt, and urea by IMCD (25, 28, 29), the documented enhancement of purinergic-driven prostanoid production in IMCD of hydrated rats may constitute a potent AVP-independent regulatory mechanism.

In recent years, the potential roles of extracellular nucleotides in the regulation of renal tubular transport, especially that mediated by COX-2 inhibition on ATPγS-stimulated PGE2 release by IMCD from hydrated rats.
of salt and water, are increasingly recognized (31). P2Y2 receptor is an extracellular nucleotide with an agonist potency order of UTP > ATP > ATPγS > 2-MeS-ATP. Pharmacological and molecular approaches localized P2Y2 purinergic receptor in the IMCD of rat (9, 17). Our previous studies documented its physiological significance in opposing the AVP-mediated water reabsorption in isolated, microperfused rat IMCD (16). We also documented its ability to induce production and release of PGE2 by rat IMCD preparations (36). PGE2 is a major prostanoid in the kidney and it interacts with four known G protein-coupled E prostanoid receptors (EP1 to EP4). Through these prostanoid receptors, PGE2 modulates renal hemodynamics and salt and water excretion (5). The functional antagonism of PGE2 on AVP-stimulated water permeability in IMCD has been known for some time (11, 25). However, only recently the cell biological mechanisms of this antagonistic effect have been unraveled. Using ex vivo preparations of renal medulla, Zelenina et al. (42) demonstrated that agonist stimulation of EP3 prostanoid receptor in IMCD causes

Fig. 6. Effect of cyclooxygenase (COX)-1 inhibition by valeroyl salicylate (Val) on ATPγS-stimulated release of PGE2 by IMCD preparations from hydrated rats. Fractions enriched in IMCD were prepared by pooling inner medullas from 4 hydrated rats. Aliquots of IMCD preparations suspended in oxygenated physiological solution were warmed to 37°C and then preincubated for 15 min with/without the addition of valeroyl salicylate to a final concentration of 30 or 300 μM. After preincubation, ATPγS was added to some of the incubations to a final concentration of 50 μM, and incubations were continued for 20 min at 37°C. Reactions were stopped by adding chilled incubation buffer. After the cellular elements were pelleted by centrifugation, the PGE2 concentrations in the supernatants were assayed by EIA and normalized to the protein contents of the incubations. Incubations were done in triplicate, and the data are presented as means ± SE. A: data as ng of PGE2 released per mg of cellular protein. B: depicts the same data as percentage of vehicle group. *Significantly different from the vehicle alone group, P < 0.001 by ANOVA. **Not significantly different from the 50 μM ATPγS group. ***Significantly different from the 50 μM ATPγS group, P < 0.001 by ANOVA.

Fig. 7. Effect of COX-2 inhibition by NS-398 on ATPγS-stimulated release of PGE2 by IMCD preparations from hydrated rats. Fractions enriched in IMCD were prepared by pooling inner medullas from 4 hydrated rats. Aliquots of IMCD preparations suspended in oxygenated physiological solution were warmed to 37°C and then preincubated for 5 min with or without the addition of NS-398 to a final concentration of 10 or 30 μM. After preincubination, ATPγS was added to some of the incubations to a final concentration of 50 μM, and incubations were continued for 20 min at 37°C. Reactions were stopped by adding chilled incubation buffer. After the cellular elements were pelleted by centrifugation, the PGE2 concentrations in the supernatants were assayed by EIA and normalized to the protein contents of the incubations. Incubations were done in triplicate, and the data are presented as means ± SE. A: data as ng of PGE2 released per mg of cellular protein. B: depicts the same data as percentage of vehicle group. *Significantly different from the vehicle alone group, P < 0.001 by ANOVA. **Not significantly different from the 50 μM ATPγS group. ***Significantly different from the 50 μM ATPγS group, P < 0.001 by ANOVA.
retention of AQP2 water channel from the apical membrane. This effectively reduces the abundance of AQP2 protein in the apical membrane, the rate-limiting barrier in the transepithelial water movement in the collecting duct.

Recently, we observed that expression of P2Y2 receptor mRNA and protein is significantly higher in the inner medulla of hydrated rats compared with the dehydrated rats (18). This prompted us to examine whether the observed increase in P2Y2 receptor expression in hydrated rats is also associated with an increased purinergic-stimulated PGE2 release by the IMCD. To address this question, we used a model of freshly prepared IMCD fractions from collagenase- and hyaluronidase-digested rat inner medullas. This is a well-characterized model for the study of hormonal response of IMCD. In our previous communications, we validated the purity and viability of the IMCD preparation (17, 19, 36). Furthermore, based on the currently available experimental data, we can safely ascertain that P2Y2 receptor is perhaps the only P2 receptor whose expression in “native rat IMCD” is characterized by molecular, pharmacological, and functional approaches (9, 16, 17). Although the presence of other P2 receptors has been reported in cultured cell lines of renal collecting duct origin, such as mIMCD-3 cells (37), their presence in native rat IMCD has not been established. Furthermore, adenosine A1 receptor has been localized and characterized in the rat IMCD (10, 38). However, the use of ATPγS, a nonhydrolyzable form of ATP, as a ligand in our experiments precluded the stimulation of functional adenosine receptors in our system.

Every renal structure produces PGE2. However, the medullary collecting duct accounts for ∼66% of the total PGE2 synthesized in the kidney. PGE2 produced in other renal structures is very low (3 to 6% of the total for each segment) (4). The release of synthesized prostaglandins from the cells, including collecting duct principal cells, is facilitated via specific prostaglandin transporters in the cell membranes (1). Prostaglandins that enter the tubular lumen are partially reabsorbed from the urine, and urinary prostaglandins probably reflect renal synthesis (8). In our study, the pattern of urinary excretion of PGE2 metabolite in normal, dehydrated, and hydrated rats has parallels to the P2Y2 receptor-stimulated PGE2 release by the IMCD in these groups. It is very striking to note the large differences between dehydrated and hydrated rats with respect to the urinary excretion of PGE2 metabolite as well as to the P2Y2 receptor-stimulated PGE2 release by the IMCD. These observations indicate that the urinary excretion of PGE2 metabolite represents the in vivo production and release of PGE2 by medullary collecting duct under the conditions of hydration and dehydration.

In our earlier study, we documented that the ATPγS-stimulated PGE2 by IMCD of normal rats was 4.3-fold over the unstimulated values after 60 min of incubation (36). In the current study, the stimulated PGE2 release by IMCD of hydrated rats was five- and sixfold over the unstimulated values after 40 and 80 min of incubation, respectively (Fig. 4). Similarly, the concentration-response curve for release of P2Y2 receptor-stimulated PGE2 by IMCD of normal rats attained a plateau over 25 μM ATPγS concentration (36). However, the corresponding concentration-response curve for the IMCD of hydrated rats showed a tendency for continued increase between 50 to 100 μM ATPγS despite a large variation at 100 μM ATPγS concentration (Fig. 5). These data indicate that hydration of rats sensitizes the purinergic signaling system in the IMCD. Such sensitization results in increased production of PGE2 when the IMCD are exposed to concentrations of extracellular ATP that are comparable to the ones found in normal rats. Based on our current and previous data (36), we predict that the set point of activity of purinergic signaling is altered in the IMCD of hydrated rats compared with normal or dehydrated rats. This lowered threshold for purinergic signaling in hydrated rats results in enhanced production of PGE2 at extracellular concentrations of ATP that are comparable to the ones seen in normal rats.

Almost every renal cell constitutively expresses COX-1. In contrast, COX-2 distribution in normal kidney is limited to only macula densa cells in the cortex and interstitial cells in the medulla. However, an increase in the medullary tonyctic, such as that seen in dehydrated conditions, is known to induce COX-2, but not COX-1, expression in the medullary collecting duct cells of rat (40). Our observation on the COX-1 activity-dependent production of PGE2 in the IMCD of normal and hydrated polyuric rats is consistent with the expression of P2Y2 receptor and the constitutive expression of COX-1, but not COX-2, in IMCD cells under these conditions. "Previous studies from our laboratories showed that concentrations of NS-398 used in the current experiments inhibited COX-2-mediated effects in cultured cortical neuronal cells (6). There is a possibility that our IMCD preparation may contain a few medullary interstitial cells, which express abundant amounts of COX-2 mRNA and protein (12); Yet, it is unlikely that these cells are stimulated by ATPγS, because our immunohistochemical studies could not detect P2Y2 receptor protein in medullary interstitial cells (17; and Kishore, unpublished observations). Our results on the lack of COX-2 activity dependency in normal and hydrated rats are also consistent with this notion. On the other hand, it is interesting to note that the basal production of PGE2 in the IMCD of dehydrated rats is low. In addition, ATPγS failed to stimulate any noticeable increase in PGE2 release, despite the fact that COX-2 expression is induced in IMCD cells by dehydration. This finding leads to two important conclusions. First, the COX-2 expression in the IMCD of dehydrated rats is not associated with production and/or release of significant amounts of PGE2. It may play a role in the survival of medullary cells under hypotonic conditions as suggested by Yang et al. (39). Second, the signal transduction from the stimulation of P2Y2 receptor to the production of PGE2 in IMCD appears to be specifically coupled to the COX-1 activity. Evidence for such functional coupling of prostanoi biosynthetic enzymes in various cells does exist (21, 24).

In conclusion, our study has important physiological significance with regard to the role of purinergic regulation of medullary collecting duct function in conditions of hydration and dehydration. This provides a basis for the operation of AVP-independent regulatory mechanisms that determine the overall function of IMCD in these conditions. Our study also emphasizes the complex nature of the interaction between the purinergic and prostanoid systems and the intracellular coupling pathways involved in such interaction.

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