Genetic deletion of the P2Y\textsubscript{2} receptor offers significant resistance to development of lithium-induced polyuria accompanied by alterations in PGE\textsubscript{2} signaling

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Submitted 8 August 2011; accepted in final form 27 September 2011

Zhang Y, Pop IL, Carlson NG, Kishore BK. Genetic deletion of the P2Y\textsubscript{2} receptor offers significant resistance to development of lithium-induced polyuria accompanied by alterations in PGE\textsubscript{2} signaling, Am J Physiol Renal Physiol 302: F70–F77, 2012. First published October 5, 2011; doi:10.1152/ajprenal.00444.2011.—Lithium (Li)-induced polyuria is due to resistance of the medullary collecting duct (mCD) to the action of arginine vasopressin (AVP), apparently mediated by increased production of PGE\textsubscript{2}. We previously reported that the P2Y\textsubscript{2} receptor (P2Y\textsubscript{2}-R) antagonizes the action of AVP on the mCD and may play a role in Li-induced polyuria by enhancing the production of PGE\textsubscript{2} in mCD. Hence, we hypothesized that genetic deletion of P2Y\textsubscript{2}-R should ameliorate Li-induced polyuria. Wild-type (WT) or P2Y\textsubscript{2}-R knockout (KO) mice were fed normal or Li-added diets for 14 days and euthanized. Li-induced polyuria, and decreases in urine osmolality and AQP2 protein abundance in the renal medulla, were significantly less compared with WT mice despite the lack of differences in Li intake or terminal serum or inner medullary tissue Li levels. Li-induced increased urinary excretion of PGE\textsubscript{2} was not affected in KO mice. However, prostanoid EP\textsubscript{3} receptor (EP\textsubscript{3}-R) protein abundance in the renal medulla of KO mice was markedly lower vs. WT mice, irrespective of the dietary regimen. The protein abundances of other EP-Rs were not altered across the groups irrespective of the dietary regimen. Ex vivo stimulation of mCD with PGE\textsubscript{2} generated significantly more cAMP in Li-fed KO mice (130%) vs. Li-fed WT mice (100%). Taken together, these data suggest 1) genetic deletion of P2Y\textsubscript{2}-R offers significant resistance to the development of Li-induced polyuria; and 2) this resistance is apparently due to altered PGE\textsubscript{2} signaling mediated by a marked decrease in EP\textsubscript{3}-R protein abundance in the medulla, thus attenuating the EP\textsubscript{3}-mediated decrease in cAMP levels in mCD.

vasopressin; aquaporin-2; ATP; prostaglandin E\textsubscript{2}; prostanoid EP\textsubscript{3} receptor

LITHIUM (Li) is still widely used in the treatment of bipolar disorder by virtue of its ability to prevent suicidal tendencies (9, 19). Despite their better tolerability, the newer drugs have not proved to be more efficacious than Li for the treatment of bipolar disorder (18). Furthermore, in recent years Li has emerged as a robust neuroprotective agent for the treatment of acute brain injury as well as chronic neurodegenerative disorders (20). However, a major limitation in chronic Li treatment in bipolar patients is the development of nephrogenic diabetes insipidus (NDI). Li-induced NDI manifests as polyuria, polydipsia, and reduced ability to concentrate urine associated with a lack of response of the medullary collecting duct to arginine vasopressin (AVP). The latter results in a marked decrease in aquaporin-2 (AQP2) water channel protein in the renal medulla (6). The molecular mechanisms of Li-induced NDI are still debatable (23). In addition to significant social inconvenience, NDI causes considerable morbidity and even mortality, especially in elderly patients. Since currently available therapeutic modalities for Li-induced NDI are not always safe (6), there is a need for continued research to understand the molecular mechanisms of Li-induced NDI with a view to find better remedial measures.

Increased production of renal PGE\textsubscript{2} has been considered as a causative factor in Li-induced NDI (24). Suppression of PGE\textsubscript{2} production in Li-induced NDI by the administration of cyclooxygenase-2 (COX-2) inhibitors was shown to improve polyuria via upregulation of the AQP2 water channel and Na-K-2Cl cotransporter (NKCC2) (12). This suggests that PGE\textsubscript{2} signaling through one or more of its receptors (EP\textsubscript{1}–EP\textsubscript{4}) may play a critical role in Li-induced NDI.

We previously identified that extracellular nucleotide (ATP/UTP)-activated P2Y\textsubscript{2}-R opposes the AVP-induced water absorption in the medullary collecting duct by decreasing cellular cAMP levels through a PKC-dependent mechanism (15). Accordingly, we and others have observed that genetic deletion of P2Y\textsubscript{2}-R significantly increases the urinary concentrating ability of the kidney by apparently sensitizing the nephron segments to circulating AVP levels (21–27). This effect on urinary concentration was associated with significantly increased protein abundances of three key AVP-regulated channels or transporters, namely, AQP2, NKCC2, and urea transporter-A (UT-A) in P2Y\textsubscript{2}-R knockout (KO) mice (21, 27). We also reported that P2Y receptors may play a role in Li-induced polyuria by enhancing the production of PGE\textsubscript{2} in medullary collecting ducts (26).

Based on our novel findings, we hypothesized that genetic deletion of P2Y\textsubscript{2}-R should ameliorate Li-induced polyuria. To address this hypothesis, we examined the development of Li-induced polyuria in wild-type (WT) and P2Y\textsubscript{2}-R KO mice, specifically probing the potential relationship between P2Y\textsubscript{2}-R and PGE\textsubscript{2} signaling in the development of the AVP-resistant state in Li-induced NDI. We show that genetic deletion of P2Y\textsubscript{2}-R significantly decreases the development of Li-induced polyuria, without altering renal accumulation of Li. Moreover, we found that this effect was independent of PGE\textsubscript{2} production, but rather related to changes in expression and signaling through prostanoid receptors.

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METHODS

Experimental animals. The animal procedures described here were approved by the Institutional Animal Care and Use Committees of the Veterans Affairs Salt Lake City Health Care System and/or the University of Utah. Breeders of mice deficient in P2Y2-R in the B6D2 genetic background were obtained from Dr. Beverly Koller (Univ. of North Carolina at Chapel Hill, Chapel Hill, NC) (3, 10). Breeding and genotypic evaluation of the mice were described previously (27). Age- and sex-matched WT and KO mice were fed normal rodent chow or rodent chow with added Li chloride (40 mmol/kg chow; MP Biomedicals, Aurora, OH) for 14 days and then euthanized. All mice had free access to drinking water during the experimental period. Twenty-four-hour urine samples were collected for 2 or 3 consecutive days before and toward the end of the experimental period. Li intake and water consumption were determined. Blood and kidney tissues were collected at necropsy. Cortical and medullary regions of the kidneys were dissected out, flash frozen, and then processed for laboratory assays.

Blood and urine analysis. Osmolalities of urine and serum samples were measured by the vapor pressure method (Wescor, Logan, UT). Serum Li levels were determined on an AVL 9180 Electrolyte Analyzer (AVL Scientific, Rosewell, GA). Urinary excretion of PGE2 metabolites and cAMP was determined by the use of commercial EIA kits (Cayman Chemical, Ann Arbor, MI).

Measurement of inner medullary tissue Li levels. Inner medullary tissue samples were homogenized in ultrapure water containing 0.5% SDS and 0.05% sodium azide. Li levels in the homogenate were determined by inductively coupled plasma-mass spectrometry (ICPMS; Exova Santa Fe Springs, CA) and were normalized to the protein contents of the homogenates. ICPMS has a detection limit of 0.06 ppm for Li, with recovery of 93% in spiked samples. Li was not detectable in the homogenization medium.

Western blotting. Processing of the kidney tissue samples and immunoblotting were done as described previously (25–27). Generation and characterization of AQP2 antibody (GN-762) was described.

Fig. 1. Lithium (Li)-induced alterations in water consumption, urine output, and urine osmolality in wild-type (WT) and P2Y2 receptor (P2Y2-R) knockout (KO) mice. Groups of mice were fed a normal diet (n = 10/genotype) or Li-added diet (n = 14/genotype) for 14 days with free access to food and drinking water. Results shown are the average of data collected on the last 2 days before euthanasia. A: water intake (ml/24 h). B: urine output (ml/14 h). C: urine osmolality (mosmol/kgH2O). Values are means ± SE. Statistical comparisons shown on the bars in A–C are by ANOVA followed by a Tukey-Kramer multiple comparison test. Also depicted (a–c) are percent changes (increase or decrease) after Li feeding in each genotype (statistical comparisons by unpaired t-test; n = 14/group). NS, not statistically significant. *P < 0.05 by Student’s t-test, but not by ANOVA.
previously (13, 25). Rabbit polyclonal antibodies for prostanoid EP$_1$, EP$_2$, EP$_3$, and EP$_\alpha$ receptors and their corresponding blocking peptides were obtained from Cayman Chemical. Quality of the tissue samples prepared was assessed by Coomassie blue staining of loading gels, and differences in protein loading, if any, were corrected by normalizing the band densities of the target proteins to that of β-actin (Biolegend, San Diego, CA).

Quantitative real-time RT-PCR assays. This was performed essentially as described previously (25, 26). cDNA was synthesized by SuperScript Reverse Transcriptase II (Invitrogen, Carlsbad, CA). Real-time PCR amplifications were carried out on the cDNA samples in the Applied Biosystems 7500 Real-time PCR System (Foster City, CA), with AmpliTaq Gold. cDNAs were amplified for 40 cycles using SYBR Green for detection. The sequences of primer pairs for the V2 receptor were forward: TCATCAGCCACCAACCA 126–143, reverse: AGATAGCAGGGCCAGTTCCAG 258–277, amplicon 151 bp. Expression of β-actin was used to normalize the expression levels of the target gene; β-actin forward: GCCAGCTTCTAGCCAGATG, 1034–1054, reverse: GCCACCG ATCCACA CAGATG, 1108–1189, amplicon 73 bp. Commercially available primers for two other mouse housekeeping genes, GAPDH and peptidylprolyl isomerase A, were utilized to cross-check the reliability of β-actin in P2Y$_2$-R KO mice (Real Time Primers, Elkins Park, PA). To verify the specificity of amplifications, PCR products were sequenced in the DNA core facility of the University of Utah and blasted in the National Center for Biotechnology Information (NCBI) database.

Determination of stimulated release of cAMP by inner medullary collecting ducts. Fractions enriched in inner medullary collecting ducts (IMCD) were prepared from freshly dissected inner medullas as described previously (25, 26). Aliquots of the fractions enriched in IMCD, after warming to 37°C, were stimulated with 10 nM desmopressin (dDAVP; Sigma, St. Louis, MO) or 0.5 μM PGE$_2$ (Cayman Chemical) in the presence of IBMX (0.5 mM) for 2 min, the reaction was stopped by adding chilled 0.1 N HCl. IMCD fragments were pelleted, and cAMP levels in the supernatants were determined using a commercial cAMP assay kit (Cayman Chemical). Protein contents of the pellets were quantified by a CoomassiePlus Protein Assay Reagent Kit (Pierce Biotechnology, Rockford, IL). Measured cAMP levels in the samples were normalized to the protein contents of the respective pellets.

Statistical analysis. Data are expressed as means ± SE. Comparisons among the means of multiple groups were made by one-way ANOVA, followed by the assessment of statistical significance by a Tukey-Kramer multiple comparison test. Differences between the means of two groups were determined by an unpaired t-test; where applicable, a Mann-Whitney nonparametric test was used. P < 0.05 was considered significant.

RESULTS

Effect of genetic deletion of P2Y$_2$-R on Li-induced NDI. Li-induced NDI is characterized by polydipsia, polyuria, and impaired urinary concentrating ability, associated with a marked decrease in the protein abundance of AQP2 water channel in the renal medulla. Data presented in Fig. 1, A–C, show that Li-induced polyuria and impaired urinary concentrating ability are significantly less in P2Y$_2$-R KO mice compared with WT mice. In addition, there was a modest decrease of polydipsia in KO mice vs. WT mice. As a result, the Li-induced alterations in urine output and urine osmolalities were significantly different in KO mice vs. WT mice, and were ∼50% less severe (Fig. 1, B and C). It should be noted that although P2Y$_2$-R KO mice can concentrate urine more than WT mice with reduced urine output, such a phenomenon is not often observed overtly but manifests when the mice are subjected to water-balance studies (21, 27), which is consistent with the data shown here for the WT and KO mice fed a normal diet.

As shown in Fig. 2, Li feeding resulted in a marked decrease in the protein abundance of AQP2 in the medulla of WT mice (76% reduction compared with normal diet-fed WT mice). Genetic deletion of P2Y$_2$-R offered significant resistance to the Li-induced decrease in AQP2 protein abundance in the medulla (55% reduction compared with normal diet-fed KO mice; P < 0.01 vs. 76% in WT mice). It should be noted that direct comparison of the AQP2 protein abundance in the medullas of normal diet-fed WT vs. normal diet-fed KO mice is not possible in the two blots shown in Fig. 2, because they were processed separately.

Effect of genetic deletion of P2Y$_2$-R on Li absorption and disposition. We investigated the possibility that the significant resistance of P2Y$_2$-R KO mice to Li-induced NDI may be due to altered Li absorption. As shown in Fig. 2, Li feeding resulted in a marked decrease in renal AQP2 mRNA expression in WT mice (55% reduction compared with normal diet-fed KO mice; P < 0.01 vs. 76% in WT mice). It should be noted that direct comparison of the mRNA and protein levels of AQP2 in the medulla of WT vs. KO mice is not possible in the two blots shown in Fig. 2, because they were processed separately.
to lower consumption of Li through food or lower absorption of Li through the gastrointestinal tract, resulting in lower blood Li levels or lower accumulation of Li in the renal medulla. As documented in Fig. 3A, the intake of Li feeding that was determined near the end of the experimental period was not significantly different between WT and KO mice. The mean terminal serum Li levels in WT and KO mice were also not significantly different (~0.6 mmol/l; Fig. 3B). Furthermore, as shown in Fig. 3C, except for one mouse, the range of distribution of tissue Li levels in the WT group is comparable to the range of distribution seen in KO mice. Despite the numerically higher mean value seen in one WT mouse, the difference in the mean tissue Li levels between the two groups was not statistically significant even when analyzed by nonparametric methods.

Effect of genetic deletion of P2Y2-R on Li-induced changes in V2 receptor expression and AVP-stimulated cAMP production by medullary collecting ducts. To gain further insights into the effect of Li feeding on the V2 receptor-cAMP-AQP2 axis in WT mice and how it is affected by the genetic deletion of P2Y2-R, we determined the relative mRNA expression of the V2 receptor and dDAVP-stimulated ex vivo cAMP production by IMCD of WT and KO mice. As shown in Fig. 4A, Li feeding in WT mice significantly decreased V2 receptor mRNA to ~50% compared with control diet feeding. mRNA expression of the V2 receptor in the medulla of normal diet-fed KO mice was ~50% less compared with the normal diet-fed WT mice, and Li feeding did not alter the mRNA levels in KO mice.

As shown in Fig. 4B, Li feeding in WT mice caused a ~30% decrease in dDAVP-stimulated cAMP production with borderline significance (P = 0.05) apparently due to the small sample size. The dDAVP-stimulated cAMP production by the IMCD of normal diet-fed KO mice was not significantly different from the corresponding value in WT mice, and Li feeding did not alter the dDAVP-stimulated cAMP production in KO mice.

Effect of genetic deletion of P2Y2-R on Li-induced alterations in urinary PGE2 and cAMP. Previously, we documented a significant increase in urinary PGE2 in a rat model of Li-induced NDI (26). Hence, we determined the urinary PGE2 excretion in both WT and KO mice fed normal or Li-added diets. As shown in Fig. 5A, Li feeding resulted in significant increases in urinary PGE2 excretion in both WT and KO mice. Furthermore, the mean urinary excretion values of PGE2 in normal diet or Li-added diet-fed KO mice were not significantly different from the corresponding values in WT mice. Thus genetic deletion of P2Y2-R apparently did not affect urinary PGE2 excretion. In parallel, we determined the urinary excretion of cAMP. As shown in Fig. 5B, urinary excretion of cAMP was not affected by genetic deletion of P2Y2-R and/or by Li feeding.

Effect of genetic deletion of P2Y2-R and/or Li feeding on prostanoid signaling. To investigate the potential effect of P2Y2-R on prostanoid signaling in the inner medulla, we determined whether lack of P2Y2-R alters protein abundances of E-prostanoid receptors (EP1, EP2, EP3, and EP4) in the renal medulla of mice fed normal or Li-added diets. In parallel, we also determined whether there are alterations in PGE2-stimulated cAMP production by the IMCD of Li-fed WT and KO mice. The protein abundances of EP1-R or EP4-R did not show alterations in WT or KO mice with or without Li feeding, and EP2-R protein was below the limit of detection (data not shown here). However, as shown in Fig. 6A, the protein abundance of EP3-R showed significant differences in the medulla. The protein abundance of EP3-R in the normal diet-fed WT mice was markedly lower compared with the normal diet-fed WT mice (29% of the mean value of WT mice). Second, modest decreases in EP3-R were also observed in both WT (33%) and KO (18%) mice after Li-feeding.

It should be noted that the ~40-kDa band of EP3-R shown here was the only band observed in Western blotting, and it was ablated when the EP3-R antibody was preadsorbed with the blocking peptide supplied by the manufacturer (Fig. 6B), thus verifying the specificity of the antibody detection of the band. The molecular mass of the band we observed (~40 kDa) was smaller than the 50- to 55-kDa species reported by a few other investigators and is likely the nonglycosylated form of the EP3-R, which is a 40-kDa protein (365 amino acid residues).

Since EP3 is associated with attenuation of cAMP signaling, we examined whether there are differences in PGE2-induced cAMP production in the medullary collecting ducts. When freshly prepared fractions of IMCD from Li-fed WT and KO mice were stimulated by PGE2, the production of cAMP was
DISCUSSION

In this study, we documented that genetic deletion of P2Y2-R offers significant resistance to the development of Li-induced polyuria, without altering blood Li levels and renal accumulation of Li, and suppression of PGE2 production. Furthermore, we showed that the observed resistance is apparently due to altered PGE2 signaling mediated by a marked decrease in EP3-R protein abundance in the medulla, thus attenuating the EP3-R-mediated decrease in cAMP levels. Incidentally, our study also revealed that genetic deletion of P2Y2-R per se results in a marked decrease in protein abundance of EP3-R in the medulla, thus uncovering a potential interaction between these two G protein-coupled receptors in the kidney. This interaction in turn is apparently responsible for the altered PGE2 signaling in Li-induced NDI, resulting in amelioration of polyuria. These novel findings may aid in the development of better therapeutic modalities.

Li-induced NDI results from the resistance of the kidney to the action of AVP. This resistance has been attributed to the increased production of renal PGE2, which is known to antagonize the effects of AVP on the collecting duct and other nephron segments (reviewed in Ref. 8). Our previous studies in rats showed that agonist activation of P2Y2-R in the medullary collecting duct has two types of effects, namely, 1) significant decrease in AVP-stimulated water reabsorption by a PKC-dependent pathway and 2) enhanced production of PGE2 (reviewed in Ref. 15). In addition, we and others documented that genetic deletion of P2Y2-R results in increased ability of the kidney to concentrate urine, despite normal circulating levels of AVP (21, 27). This is apparently due to increased sensitivity of the kidney to circulating AVP in the absence of P2Y2-R, resulting in significant increases in the protein abundances of three AVP-regulated proteins, AQ2P, NKCC2, and UT-A (21, 27). Recently, in a rat model of Li-induced NDI, we also demonstrated that enhanced purinergic signaling may play a role in the increased production of PGE2 by the medullary collecting duct. Based on these series of novel observations, we hypothesized that genetic deletion of P2Y2-R should ameliorate Li-induced NDI.

In accordance with our hypothesis, we observed that genetic deletion of P2Y2-R results in significant resistance to Li-induced NDI. This is clearly evident in the significant differences in the Li-induced increase in urine output and decrease in urine osmolality between the WT and KO mice, although the observed differences in water intake were not significant due to large variations in WT mice. These urinary parameters were ~30% more in KO mice compared with WT mice (P < 0.03: Fig. 6C).

Fig. 4. Effect of Li feeding for 14 days on the relative mRNA expression of the vasopressin V2 receptor and desmopressin (dDAVP)-stimulated ex vivo cAMP production by the inner medullary collecting ducts (IMCD) of WT and P2Y2-R KO mice. A: mRNA expression of V2 receptor relative to the expression of β-actin in WT vs. KO mice (n = 5; unpaired t-test). Total mRNA was extracted from the whole medullary tissue (outer + inner medulla) samples, reverse transcribed, and processed for real-time PCR using gene-specific primers as described in METHODS. B: dDAVP-stimulated cAMP production by the ex vivo preparations of IMCD in WT vs. KO mice (n = 5 mice/genotype). Inner medullary tissue samples (n = 5 mice/group) were pooled separately and processed for the preparation of fractions enriched in IMCD as described in METHODS. Aliquots of the IMCD fractions were warmed to 37°C and then stimulated with 10 nM dDAVP for 20 min. cAMP concentrations in the samples were determined and normalized to their respective protein contents. Values are means ± SE (3 incubations/condition) for stimulated release after subtracting the values from vehicle controls (no dDAVP). Statistics are by unpaired t-test.

Fig. 5. Effect of Li feeding on urinary excretion of PGE2 and cAMP in WT and P2Y2-R KO mice fed a normal diet or Li-added diet for 14 days. Aliquots of 24-h urine collections were processed for the determination of PGE2 metabolite or cAMP as described in METHODS. A: urinary PGE2 metabolite (ng/24 h; n = 8/group; unpaired t-test). B: urinary cAMP (nmol/24 h; n = 5–9/group).
Despite these observations, our current data do not support a role for altered ENaC expression in the observed resistance of P2Y2-R KO mice to Li-induced NDI. It is possible that genetic deletion of αENaC or complete blockade of ENaC activity by amiloride may be needed to prevent Li accumulation in the collecting duct cells.

Li-induced alterations in the expression of the vasopressin V2 receptor is an important observation in our system. Hensen et al. (7) reported decreased vasopressin V2 receptor density in Li-treated rat kidneys. In line with this report, we found decreased vasopressin V2 receptor mRNA in Li-treated WT mice. In contrast, the KO mice have low basal levels of V2 receptor mRNA, presumably to match the lack of opposing effect of P2Y2-R, and Li feeding did not alter the V2 receptor mRNA in KO mice. Despite the differences seen in dDAVP-stimulated cAMP formation, the urinary excretion of cAMP was not significantly different across the groups.

At first glance, the puzzling aspect of our study was the paradox of significant amelioration of Li-induced polyuria in P2Y2-R KO mice in the face of an unaltered Li-induced increase in PGE2 production. The fact that genetic deletion of P2Y2-R did not affect the Li-induced increase in PGE2 production suggests that P2Y receptors other than the P2Y2 subtype may also be involved in the Li-induced increase in PGE2 production. On the other hand, it is also possible that other G protein-coupled receptors might be involved in PGE2 production. Despite the fact that the magnitude of Li-induced PGE2 production is the same in WT and KO mice, what is interesting in our study is the significant resistance to Li-induced polyuria of KO mice.
To explain the paradox of significant resistance to Li-induced polyuria despite no alterations in PGE2 production, we examined the protein abundances of the four EP prostanoid receptors in KO mice to evaluate their potential for changing PGE2 signaling. We found that genetic deletion of P2Y2-R per se markedly decreased the protein abundance of EP3-R, without altering the protein abundances of EP1-R and EP4-R (EP2-R expression is very low in the kidney). EP1-R is coupled to the phosphoinositide signaling pathway, and EP2-R and EP4-R increase cellular cAMP levels. On the other hand, activation of EP3-R can decrease cellular cAMP levels (8). Hence, a marked decrease in EP3-R protein without a corresponding change in EP4-R could offset the balance between these two signaling pathways, resulting in a tilt toward the cAMP pathway. Since it is not the absolute amount of agonist that surrounds the medullary collecting ducts but the type of receptor signaling pathways active in the cells that determines the ultimate outcome, Li-induced increased production of PGE2 in WT and KO mice should have different consequences. This notion is further supported by our observation that stimulation of ex vivo preparations of medullary collecting ducts with PGE2 led to significantly more cAMP in Li-fed KO mice compared with those from Li-fed WT mice. In this context, it is interesting to note that there is a modest (33%) decrease in EP3-R protein abundance in the medulla of Li-treated WT mice, which potentially suggests a natural reaction of the kidneys to ward off the effect of Li. However, obviously that modest decrease was not sufficient to avert the effect of the Li-induced increased production of PGE2 in WT mice.

Finally, our interpretation of data on PGE2 production and prostanoid signaling in relation to urinary concentrating ability is also in line with the study by Fernández-Llama et al. (5), which showed that COX inhibitors enhanced urinary concentrating ability in part by increasing Na-K-2Cl cotransporter expression in the thick ascending limb of Henle’s loop, and this action is most likely due to elimination of an EP3-R-mediated tonic inhibitory effect of PGE2 on cAMP production. Taken together, these studies also raise an important question on the role of PGE2 in Li-induced NDI. It appears that PGE2 production may not be the causative factor in the genesis of NDI. However, its suppression by COX inhibitors upregulates the natural corrective mechanisms by eliminating tonic inhibitions and thus counteracts the effects of Li. The study by the group of Deen (17) also raised the possibility that COX2-derived prostaglandins are not necessary for Li-induced NDI development.

In conclusion, our study demonstrates that increased production of PGE2 in Li-induced NDI per se may not contribute to Li-induced polyuria, but the nature of the prostanoid signaling in the medullary collecting duct can also determine the effect of PGE2 on urinary concentration by altering cellular cAMP levels. This relationship between PGE2 and prostanoid receptor signaling in NDI is further exemplified in the absence of P2Y2-R, which incidentally resulted in a marked decrease in EP3-R protein without alterations in EP1-R or EP4-R. This apparently tilted the PGE2 signaling in Li-induced NDI in favor of increased cellular cAMP levels, despite increased PGE2 production. Further studies are required to dissect the complex interactions between purinergic and prostanoid signaling in the medullary collecting ducts in regulating water absorption in pathophysiological conditions such as NDI.

ACKNOWLEDGMENTS

The authors thank Dr. Beverly Koller for generously supplying breeders of P2Y2 receptor null and wild-type mice, Drs. Donald Kohan and Raoul Nelson for helpful suggestions, and Jeremy Judge and Craig D. Kamerath for technical assistance. Thanks are due to Dr. Jeff M. Sands for critical reading of the manuscript and helpful suggestions.

Parts of this work have been presented at the 39th and 42nd Annual Meetings of the American Society of Nephrology (October-November, 2006 and 2009, San Diego, CA) and appeared as printed abstracts in the proceedings of those meetings (11, 14).

GRANTS

This work was supported by the Department of Veterans Affairs Merit Review Project (to B. K. Kishore), the National Kidney Foundation of Utah and Idaho (to B. K. Kishore), and the resources and facilities at the VA Salt Lake City Health Care System.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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