Is There a Role for PGE2 in Urinary Concentration?

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ABSTRACT
Prostanoids are prominent, yet complex, components in the maintenance of body water homeostasis. Recent functional and molecular studies have revealed that the local lipid mediator PGE2 is involved both in water excretion and absorption. The biologic actions of PGE2 are exerted through four different G-protein–coupled receptors; designated EP1–4, which couple to separate intracellular signaling pathways. Here, we discuss new developments in our understanding of the actions of PGE2 that have been uncovered utilizing receptor specific agonists and antagonists, EP receptor and PG synthase knockout mice, polyuric animal models, and the new understanding of the molecular regulation of collecting duct water permeability. The role of PGE2 in urinary concentration comprises a variety of mechanisms, which are not fully understood and likely depend on which receptor is activated under a particular physiologic condition. EP3 and microsomal PG synthase type 1 play a role in decreasing collecting duct water permeability and increasing water excretion, whereas EP2 and EP4 can bypass vasopressin signaling and increase water reabsorption through two different intracellular signaling pathways. PGE2 has an intricate role in urinary concentration, and we now suggest how targeting specific prostanoid receptor signaling pathways could be exploited for the treatment of disorders in water balance.


The neurohypophyseal hormone, vasopressin (VP), is a major regulator of renal water excretion, predominantly through the seven trans-membrane receptor (7TMR) V2R. It binds to Gαs, inducing the cAMP second messenger system, resulting in increased NaCl absorption by the thick ascending limb and increased urea transport in the inner medullary collecting ducts (IMCDs).1–3 In addition, VP induces accumulation of the water channel aquaporin-2 (AQP2) in the rate-limiting apical plasma membrane of collecting duct (CD) principal cells,4 a process that involves a diverse range of post-translational modifications including phosphorylation of AQP2.5 Thus, VP increases the osmotic gradient for water transport and the water permeability (Pf) of the CD simultaneously.

In addition to systemic hormones, local regulatory factors play a role in the CD, of which PGE2 (Figure 1) is the focus of this review.6–9 PGE2 has a diverse range of biologic actions elicited by four different 7TMRs. The renal localization of EP receptors and intracellular signaling pathways are diverse (Table 1).10–35

Here, we propose that urinary concentration does not rely exclusively on fluctuations in plasma VP concentrations, but that the locally derived lipid mediator, PGE2, plays an important role in regulating water excretion.

PGE2 IN THE MODULATION OF URINARY CONCENTRATING MECHANISMS

The ability of PGE2 to modulate the effects of VP in the CD has been described in a range of experimental studies and discussed in previous reviews.36–39

The present focus is on understanding the mechanisms for this effect, and on recent in vivo studies elucidating the role of PGE2 in water excretion.

PGE2 decreases VP-induced increases in water permeability and AQP2 trafficking. The proposed diuretic actions of PGE2 are depicted in Figure 2. In rat kidney slices, isolated rat IMCDs, and rat IMCD primary cultures, PGE2 or the EP1/3 agonist, sulprostone, decreases VP-induced Pf and AQP2 membrane targeting without affecting cAMP levels or AQP2 phosphorylation at ser-256.40,41 Furthermore, PGE2 decreases IMCD Pf despite stimulation with the nonhydrolyzable cAMP analog 8-p-(CPT)-cAMP.42 Coincubation of sulprostone with an EP1 receptor antagonist augmented the effect on AQP2 trafficking in IMCD primary cultures.43 Increased RhoA activity and formation of F-actin were observed in this setting, which may be responsible for the observed effects on AQP2.44,45 However, no direct evidence of a causative relationship was established; rather, the events occurred in parallel. Alone, the EP1 antagonist had no effect on AQP2 targeting and did not inhibit VP-mediated AQP2 targeting, F-actin formation, or RhoA activation. It could be speculated that a variant of EP1 that can decrease EP1 and EP4 signaling46 may additionally modulate EP3, which could explain the
puzzling observations using the EP1 antagonist alone. Altogether, these studies suggest that PGE2 effects on Pf and AQP2 in VP-stimulated IMCD are elicited at a post-cAMP level.

In contrast, in rat and rabbit cortical collecting duct (CCDs), PGE2 induces a dose-dependent decrease in cAMP levels and water permeability that can be partially mimicked by sulprostone. The effect of sulprostone is eliminated by pretreatment with pertussis toxin, indicating a clear role for EP3-mediated coupling to Gi. However, amelioration of PGE2 effects requires pertussis toxin and the protein kinase C inhibitor staurosporine, suggesting that an EP receptor without affinity for sulprostone displays, as yet unknown, protein kinase C signaling.

Whether the observed differential effects are due to differential expression of EP receptors (EP3 is highly expressed in cortex and has lower expression in inner medulla, whereas EP1 shows the opposite pattern) or species differences (all reported IMCD experiments were performed in rat, whereas CCD experiments were mainly performed in rabbit, which is more prone to PGE2-induced decreases in cAMP than rat CCD) are subjects for future scrutiny. To note, EP1 receptor stimulation has never been shown to directly decrease AQP2 membrane targeting or Pf in the CD, but only in the frog urinary bladder.

A third hypothesis is that differential distribution of EP3 splice variants could play a role. EP3 exists as different splice variants in the CD, each displaying coupling to a selective G protein; most well known is Gi coupling, but Gs, Gq, and G12/13 coupling also occur. Studies using sulprostone demonstrate that in the acute setting, EP3 stimulation leads preferably to signaling through Ga in the CCD, assuming that the EP3 agonist sulprostone has equal binding affinity for all receptor subtypes. Whether expression of splice variants differs along the CD, with high expression of the Gi coupling variant in CCD and of the G12/13 variant in IMCDs is unknown. Furthermore, whether coupling undergoes

Figure 1. Synthesis of PGE2. PGE2 is a lipid mediator derived from AA, which is released from phospholipids in the nuclear membranes of most cell types. The conversion of AA to PGH2 is catalyzed by cyclooxygenase types 1 and 2 (COX-1/COX-2). COX-1 is constitutively present in many different tissues, including the kidney CD. In general, COX-2 is mainly regarded as an inducible enzyme, although it is constitutively present at specific sites in the kidney, namely the macula densa and lipid containing interstitial cells at the tip of the inner medulla. Final conversion of PGH2 into PGE2 is catalyzed by different PGE synthases, of which two are expressed in the kidney: cytosolic PGE synthase (cPGES) and microsomal PGE synthase type 1 (mPGES-1). cPGES is believed to couple mostly to COX1 and mPGES to COX-2. In line with this, cPGES is expressed only in the CD, whereas mPGES-1 is colocalized with COX-2 in interstitial cells and CD. In both cell types, COX-2 expression is upregulated in a hypertonic environment. In interstitial cells, this process involves sirtuin1 (sirt1) and in the collecting duct, the EGF receptor and mitogen-activated protein kinases.
any form of regulation so that EP3 receptor signaling could depend on the biologic context is open to speculation. Local osmolality could also play a role as hypertonicity increases the expression of EP3 (and EP4).\(^5^5\) Whether osmolality can mediate differential expression of EP3 splice variants during long-term VP stimulation would be an interesting avenue of future research.

**PGE2 IN PATHOLOGIC POLYURIC STATES**

**Lithium-Induced Polyuria**

Treatment with lithium for bipolar disorders results in nephrogenic diabetes insipidus (NDI) in approximately 20% of users. Unlike wild-type (WT) mice, in microsomal PGE synthase-1 (mPGES-1) knockout (KO) mice, lithium does not result in polyuria and downregulation of AQP2.\(^5^6,5^7\) Treatment of normal mice with a cyclooxygenase-2 (COX-2) inhibitor did alleviate lithium-induced NDI through upregulation of AQP2 and NKCC2.\(^5^8\) However, in both models, lithium uptake is a possible confounder, as this could be affected by mPGEs knockout or COX-2 inhibition, and lithium was not measured in tissue or urine. Thus, the direct involvement of PGE2 in the CD could be questioned. In contrast, although lithium treatment of mpkCCD cells decreased transcription of AQP2 and AQP2 abundance, the COX inhibitor, indomethacin, had no effect on the lithium-induced downregulation of AQP2,\(^5^9\) arguing against a role for PGE2 in lithium-induced polyuria. This idea is supported by a recent study in P2Y2 KO mice that develop a milder NDI in response to lithium treatment despite displaying increased PG levels in the urine.\(^6^0\) Interestingly, the EP3 receptor was vastly downregulated in CDs from P2Y2 KO mice, and stimulation with PGE2 \textit{ex vivo} induced a significantly higher cAMP response in lithium-treated P2Y2 KO than in lithium-treated WT. These findings suggest the CD alters its response to PGE2 in different settings, perhaps through regulation of EP receptors, and P2Y2 is a prominent regulator of EP3 abundance. Thus, evidence on PGE2 involvement in lithium-induced NDI is unclear. It appears that PGE2 may play a role \textit{in vivo}, but lithium induces downregulation of AQP2 directly in CD cells independently of PGE2.

**Postobstructive Polyuria**

Bilateral ureteral obstruction (BUO) causes a marked downregulation of AQP2 and NKCC2.\(^6^1^-6^3\) COX-2 inhibition (by NS398) was suggested to partly alleviate polyuria by increasing AQP2 protein abundance immediately after release of obstruction.\(^6^4\) However, NS398 also increased AQP2 abundance in sham operated rats, questioning the direct involvement of COX-2 in the pathogenesis of BUO. In another BUO model,\(^6^3\) COX-2 inhibition with parecoxib restored AQP2 protein levels with a minor effect.
on NKCC2 abundance. On the first day after release of obstruction, parecoxib caused a minor decrease in urine output without increasing urine osmolality, effects not observed on day 2 or going forward, suggesting that COX-2 plays no role in the long-term polyuric response. Parecoxib has even been shown to exacerbate the downregulation of AQP2 and pAQP2 in a BUO model. In general, the polyuria, downregulation, and decreased membrane targeting of AQP2, and the decreased medullary osmolality observed in several BUO models could be explained by downregulation of V2R, which parecoxib does not affect. In BUO, this could be due to local inflammatory processes, because the response is not seen in the nonobstructed kidney after unilateral ureteral obstruction.

A ROLE FOR RENAL PROSTANOIDS IN PHYSIOLOGIC WATER EXCRETION

Various animal models have elucidated a role for prostanoid signaling in the physiologic excretion of a water load. EP3 KO mice concentrate urine similar to WT mice after both water loading and water deprivation, but indomethacin administration increased urine osmolality only in WT mice. This study highlights two important points: EP3 is involved in maintaining low urine osmolality during normal conditions and EP3 KO mice compensate for the lack of the receptor through other mechanisms and thereby uphold normal or low urine osmolality. Indomethacin increases papillary osmolality in several different animal models, including water loaded Brattleboro (BB) rats that lack endogenous arginine.
vasopressin. In general, this could result from (1) PGE2 decreasing VP-induced increases in cAMP levels and subsequent NaCl transport in the medullary thick ascending limb, resulting in decreased countercurrent multiplication. This presumably occurs through EP3, (2) PGE2 increasing production of hyaluronan by interstitial cells. Hyaluronan levels in the papilla increase in response to hydration and are thought to alter the papillary interstitial matrix, causing resistance to water flow, thereby contributing to diuresis. Indomethacin would potentially block this effect, and (3) PGE2 inhibiting IMCD urea transport at a post-cAMP level.

In mPGES-1 KO mice, after an intraperitoneal injection of water, fluid excretion is severely blunted for the initial 4 hours followed by compensatory high water excretion for the remainder of the study (6 hours). This occurs alongside increased urinary PGE2 and mRNA encoding cPGES, suggesting that PGE2 is necessary for fluid excretion, but the possibility that PGE2 levels may be flow-induced should also be considered. AQP2 levels are increased in mPGES-1 KO mice at baseline, yet plasma VP levels are comparable between KO and WT mice. Similar to the conclusion drawn from EP3 KO mice, this result suggests that under baseline conditions, PGE2 plays a role in decreasing AQP2 levels. After a 24-hour water deprivation, despite blunted VP release, mPGES-1 KO mice displayed potentiated urinary concentrating ability alongside augmented increases in AQP2, UT-A1, and UT-A3 expression. Furthermore, urinary excretion of PGE2 increased in WT mice alongside kidney mRNA encoding COX-2 expression, but no change was observed in mRNA encoding mPGES. Although protein levels were not assessed, this suggests that COX-2, but not mPGES-1, is rate limiting for dehydration-induced increases in PGE2 synthesis. Any role of plasma VP in these responses should be interpreted with some caution. The measured VP levels are exceptionally high in both studies, with a 10-fold difference in levels between the studies, suggesting that acute increases in plasma VP during stress responses (due to e.g., anesthesia) could mask potential differences between WT and mPGES-1 KO mice.

A striking observation is that although mPGES-1 KO mice have an increased ability to concentrate their urine, treatment of rats with several different types of COX inhibitors reduced the concentrating ability of rats after water restriction. mPGES-1 is the main PG synthase of the papillary interstitial cells, and mPGES-1 KO mice would still synthesize PGE2 through COX-1 and cPGES in the collecting duct, where PGE2 may increase Pj. This notion is supported by the fact that the COX-2 inhibitor, meloxicam, did not induce significant changes in functional urinary parameters, but did severely downregulate AQP2, as did the non-selective COX inhibitors, indomethacin and ibuprofen. Because urinary PGE2 may be mostly derived from interstitial cell production, the lack of decreased urinary PGE2 observed in COX-inhibitor-treated rats may reflect an inability of the drug to reach these cells in the papilla, where renal blood flow is relatively low. Furthermore, the three nonsteroidal anti-inflammatory drugs used in the study can cross the blood-brain barrier and inflammatory drugs used in the study can cross the blood-brain barrier; thus, whether their effects are restricted to the CD or due to effects on the hypothalamus is an issue for speculation. Plasma VP levels measured in parallel could help to elucidate this.

AN EMERGING ROLE FOR PGE2 IN CONCENTRATING THE URINE

In rats, water restriction increases urinary PGE2 excretion and CD expression of COX-2 at levels secondary to increased papillary osmolality. All three effects are blunted in BB rats, but PGE2 excretion is restored in response to VP. Under normal conditions, BB rats also display increased COX-1, COX-2, and PGE2 in response to VP and a variety of COX-2 inhibitors severely impair urine concentration in normal rats after water deprivation. Together, these results suggest a role for PGE2 in the kidney's response to dehydration; potential mechanisms are summarized in Figure 3.

EFFECT OF EP2 AND EP4 ON URINARY CONCENTRATION

Like the V2R, EP2 and EP4 are Gs protein-coupled 7TMRs. In an inducible V2R KO mouse model, long-term treatment with the EP4 selective agonist, ONO-AE1–329 (ONO), mediated an increase in whole kidney AQP2 levels, and a single injection induced a transient increase in urine concentration. Although the Ki of ONO for mouse EP4 is approximately 10–8 M and 100 nM ONO increased IMCD Pj 2.5-fold, concentrations of 10–8 to 10–6 M were necessary for substantial increases in cAMP levels. A screening approach used in the same study indicated that no other Gs-protein–coupled receptors, other than the V2R and EP4, were present in IMCD at levels significantly greater than background. How background levels are established in this respect, remains undefined. It is possible that an additional receptor(s) could be present at low levels (below the normalized cut-off level of the screening approach) that can bind ONO at high concentrations and generate small increases in cAMP. A candidate receptor is EP2, for which ONO has a Ki of approximately 2×10–5 M in mouse, and thus fits well with the concentration curve for ONO effects on cAMP. Thus, it will be interesting to determine how EP4 increases Pj independently of cAMP and whether increased cAMP levels induced by high concentrations of ONO are mediated by EP4 or EP2.

In vitro studies in AQP2 transfected MDCK cells, which natively express EP2 and EP4, give insight into the individual roles of EP2 and EP4 on the regulation of AQP2 and hence Pj. When stimulated individually, EP2 and EP4 increased AQP2 apical membrane abundance and phosphorylation at ser-264. An EP2 receptor agonist, butaprost, increased cAMP levels and phosphorylation of AQP2 at ser-269, a site that reduces internalization of the protein, whereas an EP4 agonist (CAY10580) had no significant effect on cAMP levels indicating an
This suggests that prostanoid-induced AQP2 membrane targeting is also observed in humans, which increases the clinical relevance of these findings.

TYING UP THE ENDS: THE COMPLEX ROLE OF PGE2 IN WATER HOMEOSTASIS

How can the complex roles of PGE2 be explained? It is clear that different PGE2 receptors have different effects. However, when the CD responds to changes in homeostasis, there must be a choice of whether to respond one way or the other.

What about the shift in PGE2 effects? In rabbit cortical CDs, PGE2 at 10^{-7} M increases Pf when administered alone, but decreases the Pf induced by VP. This demonstrates unequivocally that the isolated CD itself, from the same species, in the hands of the same researchers and in the same experimental setup, shifts its response to PGE2, albeit in the presence of a supraphysiological concentration of VP. A reasonable hypothesis is that VP acutely regulates the function of EP receptors, although this has yet to receive any attention in the literature. Theoretically, this could occur through altered EP receptor membrane targeting or a shift in the downstream signaling mediated by the receptors themselves.

Promiscuity for G proteins has also been described for other 7TMRs; the β2-adrenergic receptor, where the third cytoplasmic loop and the carboxy-terminal domains are important sites for regulation of G-protein coupling and furthermore, both contain a protein kinase A (PKA) phosphorylation site. Indeed, PKA phosphorylation at the carboxy-terminal tail induces a switch in G-protein coupling from Gs to Gi. Interestingly, the main differences in structure of EP2 and EP4 are in similar loop regions: EP4 has an insertion of 25 amino acids at the third intracellular loop and contains a PKA consensus site at the carboxy-terminal tail. In addition, the Gai inhibitor, pertussis toxin, increases cAMP in EP4, but not in EP2-transfected HEK-293 cells during PGE2 stimulation. This indicates that EP4 can bind to both Gs and Gi, whereas EP2 couples only to Gs. EP4 preferably couples to Gs during PGE2 stimulation; however, we speculate that V2R-mediated PKA phosphorylation of EP4 could potentially induce a shift in EP4 receptor function from Gs to Gi coupling.

Does the mere presence of VP alter the CD response to PGE2? This is unlikely. In isolated papillae, PGE2 alone increased Pf; although the effect of VP decreased in the presence of PGE2, the combined effect of both stimuli was greater than the effect of VP alone. Critically, in addition to the relatively low concentrations of VP used in these studies, Pf was obtained in situ in intact papillae; thus, any actions of PGE2 on interstitial cells or effects of PGE2 that are due to the basic architecture of the inner medulla or the hypertonic environment would be intact. The later point is important because, in a 800 mOsm environment, increased CD adenylyl cyclase activity in response to PGE2 has been demonstrated in IMCD, suggesting that prostanoid receptors may be regulated in response to altered osmolality.

Figure 3. EP2 and EP4 increase AQP2 membrane targeting and phosphorylation in the cortical CD. EP2 and EP4 increase AQP2 apical membrane targeting, S256 phosphorylation, and S264 phosphorylation in experimental settings without the presence of VP. EP2 additionally increases S269 phosphorylation and cAMP, whereas intracellular signaling pathways of EP4 leading to AQP2 targeting and phosphorylation are currently unknown. EP4 additionally increases total kidney AQP2 protein abundance through an unknown mechanism.
FUTURE PERSPECTIVES

It is widely recognized that urine concentration is hormonally regulated by VP, which is released according to whole body hydration status. However, PGE2 can facilitate water reabsorption and water excretion by the kidney, likely dependent on which receptor is stimulated. Therefore, there is a vast array of potential therapeutic interventions that could emerge from knowing the precise details of these receptor actions. Nonsteroidal anti-inflammatory drugs are widely used for the treatment of inflammatory conditions and thrombosis and have also shown promise in prevention or treatment of colon cancer and neurologic disorders such as Alzheimer’s disease, depression, and schizophrenia,103–106 indicating that the role of PGE2 in pathophysiology is still emerging. Thus, if effects on the homeostatic mechanisms in the kidney could be established in molecular detail, any off-target effects at this site might be prevented by using selective drugs rather than the broader approach of inhibiting COX enzymes that is utilized at present. In addition, EP2 and EP4 agonists show some promise for the treatment of NDI. A promising treatment strategy of NDI could be to target EP2 or EP4 to increase CD Pf, alongside inhibition of potential PGE2 negative effects (i.e., use of EP3 antagonists).

In conclusion, multiple unanswered questions remain regarding the role of PGE2 in mediating body water homeostasis. Until very recently, the common consensus among the renal community was that PGE2 only has negative effects on CD water permeability and urine concentration. However, critical evaluation of longstanding literature and new evidence using modern day tools suggests that there are many unresolved areas for future scrutiny—and that the involvement of PGE2 in urinary concentrating mechanisms is not yet cut and dried.

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DISCLOSURES

None.

REFERENCES

22. Dasgupta S, McFerran J, Douglass JD, Alexandre D, Regardsoe EL, Korbmacher C: PGE2 stimulates Cl- secretion in murine M-1 cortical collecting duct cells in an autocrine manner. Pflugers Arch 448: 411–421, 2004
BRIEF REVIEW


77. Strosberg AD: Structure, function, and regulation of adrenergic receptors. Protein Sci 2: 1198–1209, 1993


