Dominant Role of Prostaglandin E\(_2\) EP4 Receptor in Furosemide-Induced Salt-Losing Tubulopathy: A Model for Hyperprostaglandin E Syndrome/Antenatal Bartter Syndrome

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Increased formation of prostaglandin E\(_2\) (PGE\(_2\)) is a key part of hyperprostaglandin E syndrome/antenatal Bartter syndrome (HPS/aBS), a renal disease characterized by NaCl wasting, water loss, and hyperreninism. Inhibition of PGE\(_2\) formation by cyclo-oxygenase inhibitors significantly lowers patient mortality and morbidity. However, the pathogenic role of PGE\(_2\) in HPS/aBS awaits clarification. Chronic blockade of the Na-K-2Cl co-transporter NKCC2 by diuretics causes symptoms similar to HPS/aBS and provides a useful animal model. In wild-type (WT) mice and in mice lacking distinct PGE\(_2\) receptors (EP1\(^{-/-}\), EP2\(^{-/-}\), EP3\(^{-/-}\), and EP4\(^{-/-}\)), the effect of chronic furosemide administration (7 d) on urine output, sodium and potassium excretion, and renin secretion was determined. Furthermore, furosemide-induced diuresis and renin activity were analyzed in mice with defective PGJ\(_1\) receptors (IP\(^{-/-}\)). In all animals studied, furosemide stimulated a rise in diuresis and electrolyte excretion. However, this effect was blunted in EP1\(^{-/-}\), EP3\(^{-/-}\), and EP4\(^{-/-}\) mice. Compared with WT mice, no difference was observed in EP2\(^{-/-}\) and IP\(^{-/-}\) mice. The furosemide-induced increase in plasma renin concentration was significantly decreased in EP4\(^{-/-}\) mice and to a lesser degree also in IP\(^{-/-}\) mice. Pharmacologic inhibition of EP4 receptors in furosemide-treated WT mice with the specific antagonist ONO-AE3-208 mimicked the changes in renin mRNA expression, plasma renin concentration, diuresis, and sodium excretion seen in EP4\(^{-/-}\) mice. The GFR in EP4\(^{-/-}\) mice was not changed compared with that in WT mice, which indicated that blunted diuresis and salt loss seen in EP4\(^{-/-}\) mice were not a consequence of lower GFR.

In summary, these findings demonstrate that the EP4 receptor mediates PGE\(_2\)-induced renin secretion and that EP1, EP3, and EP4 receptors all contribute to enhanced PGE\(_2\)-mediated salt and water excretion in the HPS/aBS model.


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the vasoconstriction of angiotensin II in the hyperreninemic state and maintain renal perfusion and normotension, also seen in patients with HPS/aBS (13). Recently, we demonstrated that COX-2 activity is responsible for enhanced renal PGE2 synthesis in a model of HPS/aBS (14) and that the COX-2 enzyme together with the microsomal PGE2 synthase is expressed in macula densa in patients with HPS/aBS (15,16). Furthermore, we have shown that patients who have HPS/aBS benefit significantly from selective COX-2 inhibition (13,17). After treatment with selective COX-2 inhibitors, PGE2 excretion and plasma renin activity normalized and salt and water loss decreased by approximately 50%. Despite the deleterious effect of PGE2 on HPS/aBS symptoms, the pathogenetic role of PGE2 is not understood. Although it is known that prostaglandins participate in renin-initiated counterregulation to salt loss and volume depletion, their exact role in aggravating salt wasting remains controversial. Moreover, the type of prostaglandin receptor that mediates the pathologic effect of PGE2 in HPS/aBS is presently unknown.

The activities of prostaglandins are mediated by specific G protein-coupled receptors with seven transmembrane domains (18). There are four subtypes of PGE2 receptors: EP1, EP2, EP3, and EP4. All EP receptor types are expressed within the kidney in humans (19) and rodents (20). It has been shown that PGE2 is involved in the regulation of renal blood flow, electrolyte and water reabsorption, and GFR, most likely by interaction with distinct EP receptor subtypes with specific tubular and vascular localization (19,21–27). One may speculate that the detrimental effect of PGE2 on HPS/aBS symptoms is a consequence of enhanced blood flow or glomerular filtration, reduced electrolyte reabsorption in the distal tubule, or inhibition of arginine vasopressin-stimulated water reabsorption, which suggests an important and combined role for EP1, EP2, EP3, and EP4 receptor activation.

For addressing the question of EP receptor function in HPS/aBS, the loop diuretic-treated animal represents a valuable tool. Chronic use of the diuretic furosemide, which blocks the NKA co-transporter, results in the induction of COX-2 (28) and the stimulation of renin secretion (29) and causes a clinical phenotype similar to HPS/aBS: Salt and water loss, hypotension, nephrocalcinosis, and poor growth (30,31). To elucidate further the pathogenetic role of PGE2 in HPS/aBS, we studied EP receptor function in mice deficient for a distinct subtype of the EP receptor after chronic treatment with furosemide.

Materials and Methods

Chemical Reagents

Furosemide and all other chemicals were of highest grade and were purchased from Sigma (Taufkirchen, Germany). EP4 antagonist was a gift from T. Maruyama (ONO Pharmaceuticals, Osaka, Japan).

Animals

C57BL/6 mice were purchased from Charles River (Sulzfeld, Germany). EP1-, EP2-, EP3-, and IP-deficient mice were developed as described previously (32–35). All mice were weaned at 3 wk of age and fed a standard chow diet that contained 0.9% NaCl wt/wt (Altromin, Lage, Germany). Mice were housed under controlled conditions (temperature 21 ± 1°C, 12-h light/dark rhythm). Genotypes of the mice were determined routinely by PCR analysis using oligonucleotide primers designed to detect the respective EP and IP locus and Neo cassette, as reported earlier (32–34). Experiments were conducted in female mice aged 8 to 12 wk. The State Agency Giessen approved all animal experiments, and the procedures followed were in accordance with institutional guidelines.

Animal Experiments

Furosemide was added to the drinking water at a concentration of 1 mg/ml, and mice were kept in normal cages for 4 d with standard diet and access to tap water and salt (provided as salt stone) ad libitum. Every day, the drinking volume was determined. Thereafter, animals were placed in metabolic cages for 3 d, and body weight, drinking volume, and urine production were measured. Values that were obtained at the third day were used for data analysis. Finally, animals were anesthetized and blood was obtained by cardiac puncture. Plasma was separated and kept at −80°C until determination of plasma renin activity. Kidneys were removed and were cut in longitudinal halves. One half was used for isolation of total RNA. In urine samples, electrolyte concentrations were determined by flame photometry, and excretion rates were calculated. Urinary furosemide concentration was determined as described (36). For inhibition of EP4 receptor, ONO-AE3-208 was added to the drinking water at a concentration of 0.1 mg/ml.

Ribonuclease Protection Assay for Renin and β-Actin

Renin and β-actin mRNA levels were measured by RNase protection assay as described previously (37). After phenol/chloroform extraction and ethanol precipitation, protected fragments were separated on an 8% polyacrylamide gel. The gel was dried, and bands were quantified in a PhosphorImager. Data are presented as ratio of renin signal to β-actin signal.

Measurement of Plasma Renin Concentration

Plasma renin concentration (PRC) was measured by ultramicroassay of generated angiotensin I using renin standards as described (38). Five serial dilutions from the same plasma sample were assayed in duplicate for all samples. Linearity over three serial dilutions was required to accept a value. Renin concentration is expressed in Goldblatt units (GU) compared with renin standards from the National Institute for Biologic Standards and Control (Hertfordshire, UK).

Determination of GFR

Mice were anesthetized with pentobarbital (50 µg/kg intraperitoneally) and were placed on a heated table for maintenance of body temperature at 37°C. Cannulas were placed into the trachea for facilitating breathing, the carotid artery for measurement of systemic mean arterial pressure, the jugular vein for infusion, and the urinary bladder for urine collection. After surgery, mean arterial pressure was recorded continuously. After a 60-min equilibration period, insulin in 0.9% sodium chloride solution was infused into the jugular vein by a constant infusion of 0.1 µl/min per g body wt. Blood samples were taken and urine was collected at different time points.

Statistical Analyses

Normality was assessed by D’Agostino-Pearson test. Differences between the groups were analyzed by one-way ANOVA with post Newman-Keuls multiple comparison test or Kruskal-Wallis test with Dunn’s multiple comparison test, as appropriate. P < 0.05 was considered significant.
Results
We addressed the issue of the pathogenic role of PGE2 and its receptor types in HPS/aBS by using volume-depleted mice, in which the thick ascending limb/macula densa salt transport is blocked by loop diuretic furosemide. For this purpose, controls and mice deficient in distinct PGE2 receptors were studied. Wild-type (WT) and EP receptor knockout mice could not be distinguished by visual inspection. Under normal conditions, there were no significant differences in hematocrit, plasma Na+, Cl−, K+, Ca2+, urea, and creatinine concentrations and mean arterial pressure (Table 1). They had normal body weights and kidney weights, and histologic analysis of the kidneys and hearts revealed no difference in the phenotype of EP knockout mice compared with WT mice (data not shown). The animals had free access to salt and water to compensate for their salt and water loss. Furosemide (1 mg/ml) was included in their drinking water, and we determined furosemide concentration in urine samples to ensure bioavailability of the drug in the tubular lumen. As shown in Table 2, urinary concentrations of furosemide were similar in control and knockout mice, suggesting a comparable inhibition of Na-K-2Cl co-transporter in the animals studied.

We examined urine excretion rates in WT mice and in mice deficient for each type of EP receptor. Although renal excretion of PGI2 metabolites is not increased in patients with HPS/aBS (6), we also studied mice deficient for the IP receptor as PGI2 is known to modulate renal blood flow and renin secretion. No significant difference in diuresis was observed under basal conditions between WT, EP1−/−, EP2−/−, EP3−/−, EP4−/−, and IP−/− mice (Figure 1A). The inhibition of NaCl absorption in the thick ascending limb by furosemide led to marked diuresis in all groups studied, albeit to different extents. In EP2−/− and IP−/− mice, urine production was comparable to control mouse; however, in EP1−/−, EP3−/−, and EP4−/− mice, urine volume was significantly lower (Figure 1A). Urine volume was in EP1−/− mice 68%, in EP3−/− mice 72%, and in EP4−/− mice 56% of WT mice urine output. Moreover, plasma potassium concentration was significantly lowered in furosemide-treated mice (Figure 1B). It is interesting that the decrease in EP4−/− mice was less pronounced compared with that in the other animals. In patients with HPS, the decline in plasma potassium needs careful observation, and often patients must be supplemented with potassium to avoid cardiac dysfunction (1).

The rise in urine excretion in the treated animals was matched by an increased water intake. However, the rise in drinking volume was significantly lower in WT compared with EP4−/− mice (Figure 2A). We also studied sodium and potassium excretion stimulated by furosemide. Under furosemide-free conditions, no significant difference was observed between WT and EP knockout mice (Figure 2, B and C). Similar to alterations in diuresis, we observed a strong increase in WT mice in furosemide-stimulated sodium (Figure 2B) and potassium excretion (Figure 2C) and a blunted response to furosemide in EP1−/−, EP3−/−, and EP4−/− mice. Sodium loss in EP1−/−, EP3−/−, and EP4−/− mice was limited to 74, 60, and 55% of WT mice excretion rate, respectively.

Renin activity is known to be markedly enhanced in patients with HPS/aBS. We studied PRC (Figure 3A) and expression of renal renin mRNA (Figure 3B) before and after application of furosemide in mice. Under basal condition, PRC in EP1−/−, EP2−/−, and EP3−/− mice were similar to that in WT mice. PRC in EP4−/− and IP−/− mice was slightly lower, but the difference did not reach significance. In all animals tested, furosemide caused a rise in PRC. In WT mice and in EP1−, EP2−, and EP3-deficient mice, PRC increase was similar, but in IP−/− and especially in EP4−/− mice, we observed a lower increase. Regarding renal renin mRNA expression, we observed a similar alteration after furosemide administration in all mice except EP3−/− mice. An increase in renin mRNA expression was observed in EP3−/− mice. However, this was not reflected in renin activity. Again, the weakest effect of furosemide was observed in EP4−/− mice.

To explore this issue further, we examined the role of EP4 receptor in our model by using a specific EP4 receptor antagonist. WT mice were treated with furosemide in the presence of EP4 antagonist ONO-AE3-208. Figure 4A demonstrates that the EP4 antagonist suppressed furosemide-induced diuresis significantly, and the observed urine output in WT mice was comparable to EP4−/− mice. Moreover, examination of renin mRNA expression (Figure 4B) and PRC revealed that both were suppressed by EP4 antagonist (Figure 4C) in furosemide-treated control mice.

Table 1. Blood measurements in WT and different EP receptor knockout mice

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<tr>
<td>Hematocrit (%)</td>
<td>39.3 ± 2.4</td>
<td>40.6 ± 2.9</td>
<td>38.8 ± 2.3</td>
<td>40.0 ± 1.4</td>
<td>39.8 ± 1.9</td>
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<td>Na+ (mM)</td>
<td>146.3 ± 0.6</td>
<td>148.4 ± 2.5</td>
<td>148.0 ± 0.8</td>
<td>147.8 ± 1.3</td>
<td>148.0 ± 0.6</td>
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<tr>
<td>Cl− (mM)</td>
<td>115.3 ± 2.5</td>
<td>115.3 ± 1.5</td>
<td>114.8 ± 0.8</td>
<td>114.8 ± 1.3</td>
<td>114.0 ± 0.6</td>
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<tr>
<td>K+ (mM)</td>
<td>5.08 ± 0.57</td>
<td>5.03 ± 0.19</td>
<td>5.16 ± 0.16</td>
<td>5.11 ± 0.23</td>
<td>5.10 ± 0.15</td>
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<tr>
<td>Ca2+ (mM)</td>
<td>1.43 ± 0.06</td>
<td>1.41 ± 0.06</td>
<td>1.40 ± 0.05</td>
<td>1.35 ± 0.17</td>
<td>1.21 ± 0.08</td>
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<td>Urea (mg/dl)</td>
<td>51.6 ± 5.1</td>
<td>52.5 ± 12.6</td>
<td>48.4 ± 3.9</td>
<td>53.9 ± 5.7</td>
<td>50.4 ± 4.8</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>24.84 ± 1.19</td>
<td>36.53 ± 4.23</td>
<td>32.49 ± 5.51</td>
<td>34.28 ± 2.07</td>
<td>30.87 ± 0.94</td>
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<tr>
<td>MAP (mmHg)</td>
<td>67.7 ± 5.2</td>
<td>58.6 ± 1.7</td>
<td>65.5 ± 3.5</td>
<td>60.9 ± 4.7</td>
<td>64.1 ± 4.4</td>
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*Values are means ± SEM, n = 6 to 9 for all groups. Mice were maintained on normal diet. Mice were anesthetized and blood was taken by cardiac puncture. WT, wild-type; MAP, mean arterial pressure.
Table 2. Urinary furosemide concentration*  

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<td>Furosemide (μg/ml)</td>
<td>293.4 ± 21.1</td>
<td>332.2 ± 17.8</td>
<td>257.6 ± 20.8</td>
<td>356 ± 29.0</td>
<td>284.4 ± 18.0</td>
<td>312.5 ± 25.1</td>
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*Furosemide was given for 7 d. During the last 3 d, urine from WT and different EP receptor knockout mice was collected for 24 h and furosemide concentration was determined by HPLC. Values are means ± SEM; n = 6 for all groups.

Figure 1. Urine volume (A) and plasma potassium concentration (B) by EP receptor and IP receptor knockout mice before and after treatment with furosemide. Control and knockout mice were placed in metabolic cages under basal condition for 3 d, and urine was collected. After 7 d of treatment with furosemide, urine was collected for 24 h. Blood was taken by cardiac puncture, and potassium concentration was determined in plasma samples. Data are means ± SEM (n = 12 to 14); * significant differences with P < 0.05 between the indicated groups. Not indicated in the figure, EP1−/−, EP3−/−, and EP4−/− were also significantly different from EP2−/− and IP−/−. No difference was observed between wild-type (WT) and EP4−/+ (data not shown).

A decrease in GFR may diminish the amount of fluid delivered to the distal nephron and thus limit the renal capacity to excrete water. We measured GFR in anesthetized WT and EP4−/− mice by means of inulin clearance to examine the hypothesis that a decrement in GFR in EP4-deficient mice could explain the observed diminished furosemide-induced diuresis (Figure 5). Compared with control mice, we observed no significant difference in EP4−/− mice, which indicates that suppression of furosemide effect on salt and water loss is not caused by reduced GFR in EP4−/− mice.

Discussion

We mimicked the salt-losing tubulopathy HPS/aBS by chronic administration of furosemide. Our data show an important role of the PGE2 receptor subtype EP4 in the pathogenesis of HPS/aBS. In EP4−/− mice and in WT mice that were treated with an EP4 antagonist, furosemide-induced PRC, renin expression, and urine output were significantly lower compared with those in control mice, and electrolyte excretion was reduced in EP4−/− mice. A decrease in diuresis and in electrolyte excretion but not in PRC or in renin mRNA expression was also observed in EP1 and EP3 knockout mice. However, these effects were not as strong as in EP4−/− mice. The PGE2 receptor subtype EP2 seemed to play a minor role in our model.

Considering the PGE2-mediated stimulation of renin secretion, our data point toward EP4 receptor as the transducing protein. Prostaglandins are thought to stimulate renin secretion and renin gene expression via increase of intracellular cAMP concentration (39). Among the EP receptors, EP2 and EP4 stimulate cAMP formation through Gs protein. Prostaglandins are thought to stimulate renin secretion, our data point toward EP4 receptor as the transducing protein. In support of our hypothesis, we recently observed in the two-kidney, one-clip model of renovascular hypertension, an inhibitor of angiotensin-converting enzyme, led to a similar increase in renin expression in EP2−/− compared with WT mice. Altogether, these findings are compatible with the assumption that not the EP2 receptor but most likely the EP4 receptor is important for stimulation of renin secretion mediated by the salt-depleted/macula densa–dependent pathway. In support of our hypothesis, we recently observed in the isolated, perfused kidney of EP4−/− mice the lowest stimulation of renin secretion rate by PGE2 compared with WT or EP1−/−, EP2−/−, and EP3−/− mice (42). Our data presented here indicate that at least the IP receptor may also be able to modulate renin secretion, albeit to a lesser extent compared with the EP4 receptor in our experimental setting. Similarly, in the two-kidney, one-clip model of renovascular hypertension, renin secretion was dependent on the presence of the IP receptor (43).

Under normal conditions, the activation of the renin-aldoste-
The renin system serves to counteract sodium and water loss, e.g., upon salt restriction. Secreted renin increases plasma angiotensin II concentration, which predominantly constricts efferent arterioles and medullary vasa recta, which supports GFR and lowers medullary perfusion. Furthermore, angiotensin II stimulates adrenal aldosterone release, which promotes sodium reabsorption in the distal tubule. Together, these processes serve to minimize salt loss. The molecular targets in HPS/aBS, the Na-K-2Cl co-transporter NKCC2, the ClCK channels, and the potassium channel ROMK are expressed in the cell membrane of macula densa, and they participate in detection of luminal salt concentration (44). Therefore, NKCC2, ClCK, or ROMK deficiency impairs salt detection in macula densa cells. Considering this aspect, HPS/aBS can be assumed to represent a state of massive salt restriction despite salt wasting with the consequence of renin-angiotensin system activation.

In patients with HPS/aBS, this counterregulation is overwhelmed by as-yet-unknown mechanisms leading instead to excessive salt and water loss. PGE2 has also been suggested to be involved in the control of these pathologic mechanisms. Application of COX inhibitors in patients with HPS/aBS can block pathologically increased diuresis up to 50% (13,17,45,46), and a similar decrease after COX inhibition was observed by us in furosemide-treated mice (data not shown). This indicates that NaCl and water wasting are caused by a combination of furosemide-induced blockade of sodium and chloride reab-

Figure 2. Drinking volume (A), urine sodium excretion (B), and urine potassium excretion (C) by EP receptor knockout mice before and after treatment with furosemide. Control and EP receptor knockout mice were placed in metabolic cages under basal condition for 3 d, and urine was collected. After 7 d of treatment with furosemide, urine was collected for 24 h. Data are means ± SEM (n = 12); * significant differences with P < 0.05 between the indicated groups.

Figure 3. Plasma renin concentration (PRC; A) and renin mRNA expression (B) in EP receptor and IP receptor knockout mice before and after furosemide treatment. Control and EP receptor knockout mice were placed in metabolic cages under basal condition, and blood was taken from tail vein. After treatment with furosemide, mice were anesthetized and blood was taken by cardiac puncture. Kidneys were removed, and total RNA was isolated. Data are means ± SEM (n = 12 to 14); * significant differences with P < 0.05 between the indicated groups. No difference was observed between WT and EP4+/+ (data not shown).
sorption and by additional PGE2-mediated diuretic mechanisms. Several studies indicate that PGE2 blocks sodium reabsorption most likely via the EP1 receptor and water reabsorption by inhibiting the vasopressin effect via the EP3 receptor. In human and mouse kidney, EP1 receptor has been localized to the collecting duct (19,22). Activation of EP1 receptor increases intracellular calcium levels and inhibits Na⁺/H⁺ and water reabsorption in the in vivo microperfused collecting duct (47). EP3 receptor mRNA is abundant in the thick ascending limb and collecting duct in mouse kidney (22,25). The EP3 receptor inhibits cAMP generation via a pertussis toxin-sensitive Gα-coupled mechanism, and several studies presented evidence that such type of receptor is responsible for PGE2-mediated antagonism of vasopressin-stimulated salt absorption in the thick ascending limb and water absorption in the collecting duct (48,49). These PGE2-mediated mechanisms are probably involved in our model and could explain the effects observed in EP1−/− and EP3−/− mice. In both strains, diuresis and electrolyte excretion stimulated by furosemide were blunted. However, much stronger suppression in salt and water loss could be achieved by EP4 knockout or pharmacologic EP4 receptor inhibition. Notably, the EP receptor type-dependent reductions in diuresis and electrolyte excretion most likely do not respond in an additive manner as maximal suppression of furosemide effect by approximately 50% is observed in EP4−/− mice, mirroring the effect of prostaglandin synthesis inhibition. Although not examined in our study, we assume that additional EP1 and EP3 receptor blockade will not cause a further inhibition of furosemide action in EP4−/− mice. Next to the vasopressin-dependent diuretic mechanism of PGE2, vasopressin-independent mechanisms may also exist. In nephrogenic diabetes insipidus, COX inhibitors are often used in combination with hydrochlorothiazide to ameliorate polyuria (50). The beneficial effect of COX inhibitors is seen in patients with mutations in the arginine-vasopressin receptor 2 gene as well as in the aquaporin-2 water channel gene (51). Whether under this clinical situation EP1, EP3, and/or EP4 receptors are involved needs to be clarified, but we suggest that the same PGE2-mediated mechanism may be operable in these patients.

The diuretic mechanism mediated by EP4 receptor remains to be elucidated. We can exclude the possibility that a decrement in GFR causes reduced diuresis and salt excretion, as in EP4−/− mice the inulin clearance was indistinguishable from that in WT mice and also mean arterial pressure was not different. Moreover, although the antihypertensive and diuretic actions of furosemide are known to be antagonized by inhibition of COX, several physiologic studies indicate that differences observed in hemodynamic or filtration fraction caused by COX inhibitors are not sufficient to explain decreased diuretic
and saluretic response to furosemide (52,53). According to our experience, in patients with HPS/aBS, inhibition of prostaglandin synthesis exerts no or only slight effects on filtration rate (17,46). In these patients, median creatinine clearances are within the normal range and do not differ significantly after omitting the COX inhibitor. Therefore, we assume that in patients with HPS/aBS, too, a decrease in GFR is not the responsible mechanism for the antidiuretic action of COX inhibitors.

One explanation might be found in the interaction of PGE2-stimulated EP4 receptor and electrolyte transport proteins. In accordance, PGE2 has been shown to downregulate the number of Na-K-2Cl co-transporters in medullary thin ascending limb cells (54) and to inhibit apical K+ channel activity (55), whereas COX inhibitors increased Na-K-2Cl co-transporter abundance (56). Because the NaCl transport activity by the NKCC2-ROMK system is still abrogated by tubular furosemide, other transport mechanisms have to be affected by PGE2 to explain EP4-dependent saluresis. Modulation of tubular transport proteins requires tubular expression of EP4 receptor. Localization of EP4 receptor protein in mouse still remains to be clarified, although in rat kidney, EP4 mRNA expression has been detected in the distal nephron and in the early collecting duct system (24). Yet another explanation is the well-known stimulatory effect of PGE2 on medullary perfusion, which would tend to lower concentrating ability. PGE2 directly dilates descending vasa recta, and increased medullary blood flow may contribute to enhanced salt excretion (57). This might also be a feasible explanation for the antipolyuric effect of COX inhibitors in patients who have nephrogenic diabetes insipidus. Notably, in human and rat kidney, EP4 receptor has been detected in vasa recta (19,24). Further studies are necessary to identify the EP4-dependent mechanisms to support the concept of PGE2-mediated water and electrolyte transport inhibition as a dominant facet in HPS/aBS and probably in other tubulopathies such as nephrogenic diabetes insipidus.

In summary, our data indicate that inhibition of different EP receptors can decrease furosemide-induced diuresis to different extents. This may reflect the contribution of individual EP receptors to the blockade of reabsorption and to the enhancement of secretion of salt and water. On the basis of our findings, we postulate that the EP4 receptor represents the dominant PGE2 receptor in pathogenesis of HPS/aBS. We propose EP4 receptor antagonists as valuable drugs to treat furosemide-like salt-losing tubulopathies such as HPS/aBS. The pharmacologic effect may be similar to the application of COX inhibitors. However, EP4 receptor antagonists represent a more specific approach most likely combined with fewer adverse effects. EP4 receptor agonists and antagonists also might be valuable tools to stimulate or to decrease diuresis.

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