Cyclooxygenase inhibitors increase Na-K-2Cl cotransporter abundance in thick ascending limb of Henle’s loop

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Fernández-Llama, Patricia, Carolyn A. Ecelbarger, Joseph A. Ware, Peter Andrews, Alanna J. Lee, Rachel Turner, Søren Nielsen, and Mark A. Knepper. Cyclooxygenase inhibitors increase Na-K-2Cl cotransporter abundance in thick ascending limb of Henle’s loop. Am. J. Physiol. Renal Physiol. 277 (Renal Physiol. 46): F219–F226, 1999.—Cyclooxygenase inhibitors, such as indomethacin and diclofenac, have well-described effects to enhance renal water reabsorption and urinary concentrating ability. Concentrating ability is regulated in part at the level of the thick ascending limb of Henle’s loop, where active NaCl absorption drives the countercurrent multiplication mechanism. We used semiquantitative immunoblotting to test the effects of indomethacin and diclofenac, given over a 48-h period, on the expression levels of the ion transporters responsible for active NaCl transport in the thick ascending limb. Both agents strongly increased the expression level of the apical Na-K-2Cl cotransporter in both outer medulla and cortex. Neither agent significantly altered outer medullary expression levels of other thick ascending limb proteins, namely, the type 3 Na/H exchanger (NHE-3), Tamm-Horsfall protein, or α1- or β1-subunits of the Na-K-ATPase. Administration of the EP3-selective PGE2 analog, misoprostol, to indomethacin-treated rats reversed the stimulatory effect of indomethacin on Na-K-2Cl cotransporter expression. We conclude that cyclooxygenase inhibitors enhance urinary concentrating ability in part through effects to increase Na-K-2Cl cotransporter expression in the thick ascending limb of Henle’s loop. This action is most likely due to elimination of an EP3-receptor-mediated tonic inhibitory effect of PGE2 on cAMP production.

Urinary concentration mechanism; prostaglandins; indomethacin; diclofenac

PROSTAGLANDIN H SYNTHASE (cyclooxygenase) inhibitors have been shown to increase urinary osmolality and concentrating ability (13, 20, 34). However, little is known about the molecular mechanisms responsible for this effect.

Urine is concentrated as a result of the combined functions of the collecting tubules and the loop of Henle (25), both of which are potential targets for the effects of cyclooxygenase inhibitors. The collecting duct is the site of variable osmotic equilibration with the hypertonic medullary interstitium, a process regulated through the action of vasopressin to regulate the osmotic water permeability of the collecting duct epithelium. The loop of Henle generates a high osmolality in the renal medulla through the process of countercurrent multiplication. Countercurrent multiplication in the renal medulla is dependent on net active NaCl absorption by the thick ascending limb of Henle’s loop. The apical component of NaCl absorption in the thick ascending limb is mediated by a Na-K-2Cl cotransporter, originally described by Greger and his colleagues (18, 19) and more recently identified at a molecular level by cDNA cloning (15, 31).

The abundance of the Na-K-2Cl cotransporter in the thick ascending limb is regulated in response to changes in circulating vasopressin level (24). This effect is most likely mediated through the V2 vasopressin receptor, which couples to adenylyl cyclase in the thick ascending limb via the heterotrigmeric G-protein Go, thereby increasing intracellular cAMP levels. The 5′-flanking region of the Na-K-2Cl cotransporter gene contains a cAMP regulatory element (CRE), and thus it has been proposed that the increase in protein abundance may result from cAMP-mediated increases in gene transcription rate (24). However, this hypothesis is so far untested. Prostaglandin E2 (PGE2), a major product of the cyclooxygenase pathway in the kidney, decreases cAMP levels in the thick ascending limb in vitro (39). Studies utilizing in situ hybridization (3, 4) and RT-PCR (35, 36) have demonstrated that the major PGE2 receptor in the thick ascending limb is the EP3 receptor. The EP3 receptor couples to adenylyl cyclase via the heterotrigmeric G protein, Go, which inhibits cAMP production (5). Thus endogenously produced PGE2 in the kidney would be predicted to have an inhibitory effect on thick ascending limb cAMP levels and, based on the hypothesized role of cAMP to increase Na-K-2Cl cotransporter expression in the thick ascending limb, would be expected to provide a tonic inhibitory influence on Na-K-2Cl cotransporter expression. Consequently, on the basis of existing evidence, it can be predicted that inhibitors of cyclooxygenase would increase Na-K-2Cl cotransporter expression, an effect which could help to explain the ability of cyclooxygenase inhibitors to increase concentrating ability. Thus our hypothesis is that long-term inhibition of prostaglandin synthesis may increase the expression level of the Na-K-2Cl
cotransporter in the thick ascending limb of Henle’s loop. To address this hypothesis, we have employed antibodies to the apical Na-K-2Cl cotransporter (and other thick ascending limb transporters) in semiquan-
titative immunoblotting studies of rats treated with the prostaglandin synthase inhibitors, indomethacin and diclofenac.

METHODS

Animal protocols. Pathogen-free male Sprague-Dawley rats (200–300 g body wt; National Cancer Institute Breeding Facility, Frederick, MD) were used. Initially, all rats were maintained in filter-top microisolator cages with autoclaved feed and bedding, and free access to drinking water. During the course of the experiments, rats were singly housed in metabolic cages to allow collection of daily urine samples and determination of daily water intake. In addition, the rats were weighed daily.

Three protocols were executed. The first two protocols assessed the effects of two different cyclooxygenase inhibitors, indomethacin and diclofenac, on protein abundance in the thick ascending limb. The third protocol evaluated the ability of the PGE analog, misoprostol, to reverse the effects of cyclooxygenase inhibition by indomethacin.

In the first protocol, rats were randomly divided into indomethacin- or vehicle-treatment groups. In the indomethacin treatment group, rats received indomethacin (Sigma, St. Louis, MO) at 5 mg/kg every 12 h intraperitoneally over a period of 48 h. The indomethacin was dissolved in 10 mM Na₂CO₃ solution following a standard procedure (32). The control rats were injected with saline alone. Rats were killed 48 h after the injection.

In the third protocol, rats were randomly divided into two groups: the indomethacin alone and indomethacin plus misoprostol. Misoprostol (Cayman Chemical, Ann Arbor, MI), a PGE₁ agonist with broad receptor selectivity but with high affinity for the EP₃ receptor (2), was dissolved in DMSO (ATCC, Rockville, MD). In the indomethacin plus misoprostol group, rats received four intraperitoneal injections of indomethacin (5 mg/kg every 12 h over a period of 48 h) as described above, plus six intraperitoneal injections of misoprostol (300 µg·kg⁻¹·day⁻¹ administered every 8 h over a period of 48 h). In the indomethacin alone group, rats received the same intraperitoneal injections of indomethacin (5 mg/kg every 12 h over a period of 48 h), while receiving six intraperitoneal injections of vehicle (DMSO) in lieu of the misoprostol.

Animals used for immunoblotting studies were killed by decapitation, and trunk blood was collected. Serum was separated and saved at −20°C for analysis. Kidneys were removed for immunoblotting as described below. All animal experimentation described in this article was approved by the NHLBI Animal Care and Use Committee (protocol no. 5-K-E-1).

Urinary concentrating capacity. One set of indomethacin-treated and vehicle-treated rats was utilized for measurement of urinary concentrating capacity. For this, the rats were deprived of water for 18 h and then were given an injection of 1-deamino-[8-D-arginine]-vasopressin (DDAVP, 1 nmol/100 g body wt im; Rhone-Poulenc Rorer, Collegeville, PA), a V2 receptor-selective vasopressin agonist. Spontane-
ously voided urine was collected 60–90 min after the DDAVP injection for determination of osmolality using a vapor-pressure osmometer (model 5100C; Wescor, Logan, UT).

Blood and urine analyses. Urine and serum osmolalities were measured using a vapor-pressure osmometer (model 5100C; Wescor). Urine sodium was also determined (Monarch 2000; Instrumentation Labs, Walham, MA).

Rabbit polyclonal antibodies prepared against the thick ascending limb isoform of the Na-K-2Cl cotransporter have been described previously (10, 24). They were raised against carrier-conjugated synthetic peptides corresponding to two different regions in the amino-terminal tail. Another polyclonal antibody was also prepared against the sodium/hydrogen ion exchanger isoform 3 (NHE-3). It was raised to a region in the carboxy-terminal tail of the NHE-3. Specificity of the antibodies has been demonstrated by showing unique peptide-ablatable bands on immunoblots and a unique distribution of labeling by immunohistochemistry and immunoelectron microscopy. These antibodies were affinity purified against the immunizing peptides for use in these studies.

A mouse monoclonal antibody against Na-K-ATPase α₁-subunit (Upstate Biotechnology, Lake Placid, NY), a rabbit polyclonal antibody against Na-K-ATPase β₁-subunit (Upstate Biotechnology), and an affinity-purified antibody to Tamm-Horsfall protein (21) were also used (a gift from R. Hoyer).

Kidney dissection and tissue preparation for immunoblotting. Immediately after killing each rat, we rapidly removed both kidneys and washed these briefly in ice-cold isolation buffer (described below). The right kidneys were dissected to obtain the inner stripe of the outer medulla and the cortex. The left kidney was homogenized intact to prepare a “whole kidney” preparation. The tissue was initially homogenized for 15 s using a tissue homogenizer (Omni 2000 fitted with a micro-sawtooth generator) in ice-cold isolation solution containing 250 mM sucrose/10 mM triethanolamine (Calbio-
chem, La Jolla, CA) with 1 µg/ml leupeptin (Bachem California, Torrance, CA) and 0.1 mg/ml phenylmethylsulfonyl fluoride (US Biochemical, Toledo, OH). The total protein concentration in outer medulla, cortex, or whole kidney homogenates was measured using the Pierce BCA protein assay reagent kit (Pierce, Rockford, IL). The whole homoge-
genates from the inner stripe of the outer medulla and cortex were used to study the specific regional expression of the different proteins, whereas the left kidney whole homogenate was used to study protein expression in the whole kidney.

Electrophoresis and immunoblotting. Proteins were solubi-
lized at 60°C for 15 min in Laemmli sample buffer. SDS-
PAGE was performed on 6%, 7.5%, or 12% polyacrylamide minigels (Bio-Rad Laboratories, Hercules, CA), loaded with equal amount of protein per lane. For each set of samples, an initial gel was stained with Coomassie blue to confirm that equal loading had been achieved as described previously (38). Representative bands were quantified by laser densitometry (Molecular Dynamics model PDS1-P90, ImageQuant v4.2 software), assuring that loading did not differ for any sample by more than 10% from the mean. For immunoblotting, the proteins were transferred from an unstained gel electropho-
retically to nitrocellulose membranes (Bio-Rad Laboratories). After being blocked with 5 g/dl nonfat dry milk for 30 min, the blots were probed with the respective antibodies for 24 h at 4°C. After washing with blot wash buffer containing 150 mM NaCl, 50 mM sodium phosphate, and 50 mg/dl of Tween 20 (J. T. Baker, Phillipsburg, NJ), the membranes were exposed to secondary antibody (donkey anti-rabbit immunoglobulin G conjugated with horseradish peroxidase, Pierce no. 31458,
diluted 1:5,000; goat anti-mouse immunoglobulin G conjugated with horseradish peroxidase, Pierce no. 31434, diluted 1:5,000) for 1 h at room temperature. Sites of antibody-antigen reaction were visualized using luminal-based enhanced chemiluminescence (Supersignal Substrate, Western Blotting, Pierce no. 34080; or KPL LumiGLO substrate kit for chemiluminescent detection of horseradish peroxidase-labeled reagents, Kirkegaard & Perry Laboratories). The relative expression levels of a given protein in different groups of rats were quantified by densitometry (Molecular Dynamics model PD51-P90, ImageQuaNT v4.2 software) using films exposed sufficiently long so that the relation between protein amount and band density was approximately in the linear range as described (11, 27). The band density values were normalized by dividing by the mean value for the controls and multiplying by 100%.

Presentation of data and statistical analyses. Quantitative data are presented as means ± SE. Statistical comparisons were accomplished by unpaired t-test (when variances were the same) or by Mann-Whitney rank-sum test (when variances were significantly different between groups). P < 0.05 was considered statistically significant.

RESULTS

Effect of indomethacin on thick ascending limb transporter expression. To assess possible effects of indomethacin administration on thick ascending limb ion transporter expression, rats were prepared for immunoblotting studies. In these animals, there were no differences between the indomethacin-treated group and the control group with regard to body weight (indomethacin-treated rats, 237 ± 3.2; control, 233 ± 2.2 g), water intake (indomethacin-treated rats, 30.3 ± 2.0; control, 29 ± 1.3 ml/day), urinary volume (indomethacin-treated rats, 12.6 ± 1.7; control, 12.2 ± 0.66 ml/day), urinary sodium concentration (indomethacin-treated rats, 1,436 ± 122; control, 1,439 ± 107 mosmol/kgH2O), urinary sodium concentration (indomethacin-treated rats, 101.5 ± 9.7; control, 91.4 ± 8.3 meq/l), or serum osmolality (indomethacin-treated rats, 291 ± 1.6; control, 290 ± 1.3 mosmol/kgH2O).

Immunoblots comparison thick ascending limb ion transporter expression in indomethacin-treated and control rats are shown in Figs. 1–3. As illustrated in Fig. 1, and as hypothesized in the introduction, the indomethacin-treated rats displayed a significant increase in Na-K-2Cl cotransporter abundance in both outer medulla (normalized band densities: control, 100 ± 7%; indomethacin-treated rats, 173 ± 18%, P < 0.01) and in cortex (normalized band densities: control, 100 ± 19%; indomethacin-treated rats, 162 ± 11%, P < 0.05). These observations were reproducible with multiple repeat immunoblots with the same samples and in an additional set of rats (data not shown).

To determine whether the indomethacin-induced increase in Na-K-2Cl cotransporter expression in the rat outer medulla was selective for the Na-K-2Cl cotransporter or was part of a generalized increase in expression of apical plasma membrane proteins of the thick ascending limb, additional immunoblots were run for the type 3 Na/H exchanger (NHE-3) and Tamm-Horsfall protein, using the same samples from the outer medulla as those used for Fig. 1. These immunob-
The urinary osmolality in the indomethacin-treated rats was significantly higher, 2,626 ± 105 mosmol/kgH2O, but there were differences in body weight (diclofenac-treated rats, 220 ± 3.7 g; control, 259 ± 6.3 g), water intake (diclofenac-treated rats, 33.9 ± 3.0 ml/day; control, 64 ± 2.9 ml/day), and urinary sodium concentration (diclofenac-treated rats, 11.4 ± 3.8 meq/l; control, 99.2 ± 10.7 meq/l).

Figure 4 shows an immunoblot for the thick ascending limb Na-K-2Cl cotransporter from whole kidney homogenate in diclofenac-treated and control rats. There was a 2.5-fold increase in Na-K-2Cl cotransporter expression in the whole kidney homogenates from the diclofenac-treated rats compared with the vehicle controls (normalized band densities: control, 100 ± 30%; diclofenac-treated rats, 258 ± 34%; P < 0.01). Furthermore, an increase in Na-K-2Cl abundance was observed both in the inner stripe of the outer medulla (normalized band densities: control, 100 ± 30%; diclofenac-treated rats, 315 ± 52%; P < 0.05) and in cortex (normalized band densities: control, 100 ± 59%; diclofenac-treated rats, 495 ± 162%; P < 0.05) (immunoblots not shown).

Additional immunoblots were run for NHE-3 and Tamm-Horsfall protein, using the same samples from the inner stripe of the outer medulla as those used for Fig. 4. These immunoblots are shown in Fig. 5. Again, as seen previously in the indomethacin experiments, the abundances of these two apically located proteins were not affected by diclofenac administration. For NHE-3 (Fig. 5A), the normalized band densities were:

![Immunoblot](image)

**Fig. 3.** Effect of indomethacin treatment on expression of α1-subunit (top) and β1-subunit (bottom) of Na-K-ATPase in rat renal outer medulla: immunoblots of same outer medullary homogenates from Sprague-Dawley rats as used for Figs. 1–2. Top: blot was loaded with 2 µg total protein/lane and was probed with mouse monoclonal anti-α1-subunit antibody. Band density for anti-α1-subunit of Na-K-ATPase was not significantly changed by indomethacin treatment (see text). Bottom: blot was loaded with 15 µg total protein/lane and was probed with rabbit polyclonal antisem to β1-subunit of Na-K-ATPase. Band density was not significantly changed by indomethacin treatment (see text).

![Immunoblot](image)

**Fig. 4.** Effect of diclofenac treatment on expression of Na-K-2Cl cotransporter in rat kidney: immunoblot of whole kidney homogenate in diclofenac-treated and control rats. Blot was loaded with 10 µg total protein/lane and was probed with rabbit polyclonal anti-Na-K-2Cl cotransporter antibody. Band density for Na-K-2Cl cotransporter protein was significantly increased by diclofenac treatment (see text).

1 Among renal tubule segments, Na-K-ATPase activity is highest in the outer medullary thick ascending limb (25), which accounts for ~90% of the total Na-K-ATPase activity in the inner stripe of the outer medulla. Thus changes in Na-K-ATPase expression in the inner stripe portion of outer medulla are most likely due to changes in its expression in the thick ascending limb. Consequently, we utilize total abundance of the α1-subunit of the Na-K-ATPase in the inner stripe of outer medulla as a measure of thick ascending limb expression as done previously (14).
Band density was not significantly changed by diclofenac treatment (see text). Blot was loaded with 15 µg total protein/lane and was probed with rabbit polyclonal anti-NHE-3 antibody. Band density for NHE-3 protein was not significantly altered by diclofenac treatment (see text).

There was no change in the expression level of the α1- and β1-subunits of the Na-K-ATPase (normalized band densities: control, 100 ± 11%; diclofenac-treated rats, 95 ± 30%; NS) or the β1-subunit (normalized band densities: control, 100 ± 25%; diclofenac-treated rats, 122 ± 11%; NS) in the diclofenac-treated rats compared with the control rats.

Effect of EP receptor agonist on Na-K-2Cl cotransporter expression. The effect of cyclooxygenase inhibitors on Na-K-2Cl cotransporter expression is presumably due to reduced levels of PGE2 in renal tissue. To address this possibility more directly, we have tested the effects of misoprostol, a PGE agonist with broad receptor selectivity but with highest affinity for the EP3 receptor (2), on Na-K-2Cl cotransporter expression in the setting of indomethacin treatment. Figure 7 shows a semiquantitative immunoblot comparing Na-K-2Cl cotransporter expression from outer medullary homogenates from rats given indomethacin alone vs. rats treated with indomethacin plus misoprostol. Misoprostol administration resulted in a significant decrease in Na-K-2Cl cotransporter expression (normalized band densities: indomethacin alone, 100 ± 13%; indomethacin plus misoprostol, 66 ± 5%; P < 0.05). Thus misoprostol reversed the effect of the cyclooxygenase inhibitor indomethacin on Na-K-2Cl cotransporter abundance.

DISCUSSION

The present studies were done to test the effects of cyclooxygenase inhibitors on the abundance of the ion transporter proteins in the thick ascending limb of Henle’s loop with a view toward understanding how these agents enhance renal concentrating capacity. We used two nonselective cyclooxygenase inhibitors that are frequently used clinically as antiinflammatory agents, namely, indomethacin and diclofenac. Both agents resulted in a marked selective increase in the abundance of the Na-K-2Cl cotransporter of the thick ascending limb, which mediates apical Na-CI entry in this segment and is thus centrally involved in the active transport process that drives countercurrent multiplication. Expression levels of NHE-3, Tamm-Horsfall protein, and the α1- and β1-subunits of the Na-K-ATPase were unaffected. Indomethacin- or diclofenac-treated rats, 79 ± 36% (NS). For Tamm-Horsfall protein, the normalized band densities were: control, 100 ± 4%; diclofenac-treated rats, 102 ± 10% (NS).

Figure 6 shows the effect of diclofenac administration on the expression level of the α1- and β1-subunit of Na-K-ATPase in the inner stripe of the outer medulla. There was no change in the expression level of the α1-subunit of Na-K-ATPase (normalized band densities: control, 100 ± 1%; diclofenac-treated rats, 102 ± 11% (NS)).

Figure 7 shows the effect of misoprostol administration on Na-K-2Cl cotransporter expression in rat renal outer medulla. Immunoblot of outer medullary homogenates from Sprague-Dawley rats as used for Fig. 5. Top: blot was loaded with 10 µg total protein/lane and was probed with rabbit polyclonal anti-NHE-3 antibody. Band density for NHE-3 protein was not significantly altered by diclofenac treatment (see text).

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Figure 6 shows the effect of diclofenac administration on the expression level of the α1- and β1-subunit of Na-K-ATPase in the inner stripe of the outer medulla. There was no change in the expression level of the α1-subunit of Na-K-ATPase (normalized band densities: control, 100 ± 1%; diclofenac-treated rats, 102 ± 11% (NS)). For Tamm-Horsfall protein, the normalized band densities were: control, 100 ± 4%; diclofenac-treated rats, 102 ± 10% (NS).

Figure 7 shows the effect of misoprostol administration on Na-K-2Cl cotransporter expression in rat renal outer medulla. Immunoblot of outer medullary homogenates from Sprague-Dawley rats as used for Fig. 5. Top: blot was loaded with 10 µg total protein/lane and was probed with rabbit polyclonal anti-NHE-3 antibody. Band density for NHE-3 protein was not significantly altered by diclofenac treatment (see text).
fenac-induced increases in Na-K-2Cl cotransporter expression can be expected to enhance the maximum rate of active NaCl absorption in the thick ascending limb and hence to increase the accumulation of NaCl in the medullary interstitium. This in turn would increase osmotic water absorption from the renal collecting ducts. Although these results provide evidence that cyclooxygenase inhibition has important effects on the thick ascending limb, they do not address whether effects at other nephron sites may participate in the process by which cyclooxygenase inhibitors increase concentrating ability and renal water absorption.

The major active product of the cyclooxygenase pathway in the kidney is believed to be PGE2 (26), although other products of the cyclooxygenase pathway could be postulated to have regulatory actions in the thick ascending limb and elsewhere. To address whether the effects of indomethacin and dexamethasone may be attributed to PGE2, we employed the EP receptor agonist, misoprostol (a PGE agonist with broad receptor selectivity but with highest affinity for the EP3 receptor; Ref. 2). We found that in indomethacin-treated rats, misoprostol decreased Na-K-2Cl cotransporter expression, partially reversing the stimulatory effect of indomethacin. These results suggest that the effect of indomethacin (and presumably dexamethasone) to increase Na-K-2Cl cotransporter expression could be a result of elimination of a tonic action of PGE2, binding to EP3 receptors, to decrease Na-K-2Cl cotransporter expression. This conclusion is consistent with the previous demonstration by in situ hybridization (3, 4) and RT-PCR (35, 36) techniques that the EP3 subtype of PGE receptor is expressed in the thick ascending limb. Intracellular signaling mediated by the EP3 receptor is believed to be chiefly via coupling to adenyl cyclase through the inhibitory heterotrimeric G protein, Gi (5), and hence PGE2 would be expected to act on the thick ascending limb to decrease intracellular cAMP levels. Indeed, such an effect of PGE2 has been demonstrated in microdissected thick ascending limb segments (39). These results are therefore compatible with our hypothesis that cAMP is an important regulator of Na-K-2Cl cotransporter expression and that the effect of vasopressin and restriction of water intake to increase Na-K-2Cl cotransporter expression is mediated by chronic increases in intracellular cAMP levels (24).

It should be recognized that misoprostol is “selective,” but not “specific” for the EP3 receptor. Thus we cannot exclude possible effects of PGE3 or other cyclooxygenase products on Na-K-2Cl cotransporter expression via other receptors. EP1 receptors, which activate phospholipase C-β through the heterotrimeric G protein, Gq (37), are not thought to be expressed in the thick ascending limbs. Nevertheless, recent studies from the laboratory of Good (16) suggest that PGE2 activates protein kinase C in the thick ascending limb, an effect usually associated with activation of phospholipase C. At this point, it is not known whether protein kinase C activation enhances Na-K-2Cl cotransporter expression in the thick ascending limb.

Long-term and short-term regulation of concentrating ability. The present results are compatible with the view that cyclooxygenase products play an important long-term regulatory role on thick ascending limb Na-K-2Cl cotransporter expression, which in turn may contribute to the overall regulation of urinary concentrating ability and the regulation of water excretion. Previous studies have demonstrated that the urinary concentrating process is subject to long-term regulatory processes by which sustained decreases in water intake result in adaptation of the kidney, such that concentrating ability is enhanced to a degree greater than achievable through a short-term increase in circulating vasopressin concentration (12, 22). The long-term enhancement of concentrating ability has been demonstrated to be due in part to effects in the collecting duct to increase the expression levels of the apical water channel, aquaporin-2 (8, 38), and the basolateral water channel, aquaporin-3 (9, 38). This long-term regulatory process couples with the short-term action of vasopressin to stimulate trafficking of aquaporin-2 to the plasma membrane (29) in mediating the overall regulation of water transport in the collecting duct. In addition, similar long-term effects of vasopressin have been demonstrated in the thick ascending limb of Henle’s loop. Specifically, long-term increases in circulating vasopressin level have been demonstrated to be associated with increased expression of the Na-K-2Cl cotransporter (24) and increases in transepithelial chloride transport in isolated perfused thick ascending limb (1). This long-term regulatory process couples with the previously demonstrated short-term action of vasopressin to activate the Na-K-2Cl cotransporter (28) to mediate overall regulation of thick ascending limb NaCl absorption and countercurrent multiplication.

Based on the results of the present study and on previous observations, it appears that PGE2, like vasopressin, has both long- and short-term regulatory actions in the thick ascending limb. Specifically, studies by Stokes (33) in the late 1970s demonstrated that PGE2 has an inhibitory effect on NaCl absorption in isolated perfused thick ascending limbs from rabbits, an observation which has been confirmed in mouse (6) and rat (17). This effect is mediated by an inhibitory G protein (7), consistent with the role of the EP3 receptor. This inhibitory effect is seen within a few minutes of exposure of the thick ascending limb epithelium to the PGE2, a response which is probably too rapid to be caused by increased abundance of the Na-K-2Cl cotransporter. Indeed, it has recently been suggested that short-term regulation of the Na-K-2Cl cotransporter occurs via trafficking of intracellular vesicles containing the transporter to the plasma membrane (30). Recent studies in cultured mouse medullary thick ascending limb cells suggest that the short-term inhibitory effect of PGE2 is a result of an action of PGE2 to regulate Na-K-2Cl cotransporter activity (23). Thus there appear to be dual mechanisms for regulation of thick ascending limb Na-K-2Cl cotransport that are effective over different time frames. Thus far, it is not known...
whether the long-term regulation of Na-K-2Cl cotransporter abundance by vasopressin and PGE2 is a result of altered Na-K-2Cl cotransporter mRNA levels or whether it could be due to alternative mechanisms such as translational regulation or regulation of Na-K-2Cl cotransporter protein degradation.

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