Vasopressin-mediated regulation of epithelial sodium channel abundance in rat kidney

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Received 2 November 1999; accepted in final form 8 February 2000

Ecelbarger, Carolyn A., Gheun-Ho Kim, James Terris, Shyama Masilamani, Carter Mitchell, Ivan Reyes, Joseph G. Verbalis, and Mark A. Knepper. Vasopressin-mediated regulation of epithelial sodium channel abundance in rat kidney. Am J Physiol Renal Physiol 279: F46–F53, 2000.—Sodium transport is increased by vasopressin in the cortical collecting ducts of rats and rabbits. Here we investigate, by quantitative immunoblotting, the effects of vasopressin on abundances of the epithelial sodium channel (ENaC) subunits (α, β, and γ) in rat kidney. Seven-day infusion of 1-deamino-[8-D-arginine]-vasopressin (dDAVP) to Brattleboro rats markedly increased whole kidney abundances of β- and γ-ENaC (to 238% and 288% of vehicle, respectively), whereas α-ENaC was more modestly, yet significantly, increased (to 142% of vehicle). Similarly, 7-day water restriction in Sprague-Dawley rats resulted in significantly increased abundances of β- and γ-ENaC, but no significant change in α-ENaC. Acute administration of dDAVP (2 nmol) to Brattleboro rats resulted in modest, but significant, increases in abundance for all ENaC subunits, within 1 h. In conclusion, all three subunits of ENaC are upregulated by vasopressin with temporal and regional differences. These changes are too slow to play a major role in the short-term action of vasopressin to stimulate sodium reabsorption in the collecting duct. Long-term increases in ENaC abundance should add to the short-term regulatory mechanisms (undefined in this study) to enhance sodium transport in the renal collecting duct.

imunoblotting; sodium transporters; collecting duct; Brattleboro rat; aldosterone

THE POSTERIOR-PITUITARY HORMONE, vasopressin, has been shown to increase both sodium (9, 18, 20, 40, 41, 47, 51) and water reabsorption (19, 33) in the kidney collecting duct of rats and/or rabbits. Its role in water reabsorption has been well described. Briefly, vasopressin, acting through the vasopressin V2 receptor, can increase water permeability of the collecting duct both by acutely regulating trafficking of aquaporin-2 (22, 35) and, over a longer time frame, by increasing transcription and thus influencing the total number of aquaporin-2 (10, 23, 36, 49) and aquaporin-3 (13, 14, 49)

water channel molecules in the principal cells of the collecting duct.

The role of vasopressin in the regulation of sodium balance is not as clear. Several investigators have shown, in acute studies, that in vitro application of vasopressin will increase sodium transport in perfused cortical collecting ducts (CCD) from rat (9, 20, 40, 41, 45, 51) or rabbit, (9, 18) as well as in primary rabbit CCD suspensions (7) and in several cell lines such as A6 cells (cultured toad kidney cells) (3, 52) and M-1 cells (derived from mouse CCD) (34). Furthermore, in perfused tubules the effect was additive to, and thus independent from, the increase in transport observed with aldosterone (9, 2, 41). Furthermore, in several of the above perfused-tubule studies, it was shown that without pretreatment of animals with mineralocorticoids, sodium transport in response to vasopressin was nearly imperceptible in the CCDs (41, 51). Less work has been done examining the chronic effects of vasopressin on sodium transport in the kidney. Djelidi et al. (11) have reported that chronic exposure to vasopressin will increase 22Na influx in RCCD1 (rat CCD) cells. However, the effects of chronically elevated vasopressin levels, in vivo, on sodium transport capacity in this segment have not been studied.

Sodium transport across the apical membrane of the cortical and outer medullary collecting duct occurs primarily through the amiloride-sensitive epithelial sodium channel (ENaC) (2). This channel, in the kidney, is a hetero-multimer made up of three distinct, yet homologous subunits: α, β and γ. Cloning of the three subunits from rat colon (6, 8) has made it possible to study the molecular regulation of these proteins. We, as well as others (11, 12, 42), have made antibodies against each of the subunits. Our antibodies are peptide-derived, polyclonal antibodies directed against a region in the carboxy tail of β- and γ-ENaC and against a region in the amino tail of α-ENaC (28). These antibodies are unique in that they are sufficiently sensitive to detect ENaC in native tissues by immunoblotting.

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Vasopressin V2 receptor mRNA has been localized, by both in situ hybridization (38) and RT-PCR (19, 25, 48), to the same tubule segments that express ENaC, that is, the connecting tubule and the collecting duct. The V2 receptor is coupled to adenylyl cyclase through Gsα activation in these cells. The long- and short-term antidiuretic actions of vasopressin are found to be primarily mediated by increased cellular cAMP levels. Chronic exposure to vasopressin is thought to lead to increased expression of aquaporin-2 protein via potentially multiple effects on regulatory motifs (or elements) in the 5′-flanking region of the gene. One such element is the CRE (cAMP regulatory element), which increases transcription rates for this protein (29). Interestingly, a CRE has also been found in the 5′-flanking region of γ-ENaC (50). Moreover, Djelidi et al. (11) have shown vasopressin-stimulated increases in transcription and translation of both γ- and β-ENaC in RCCD1 cells. Thus the purpose of these studies was to evaluate the effects of both acute and chronic elevation of circulating levels of vasopressin [or the V2-specific receptor agonist, 1-deamino-(8-D-arginine)-vasopressin (dDAVP)] on α-, β-, and γ-ENaC subunit abundances in the rat kidney by quantitative immunoblotting.

**METHODS**

**Animals and Study Design**

For these experiments, four different groups of animals were studied. In the first, 12 male Brattleboro rats (250 g), under light methoxyflurane (Metofane, Schering-Plough Animal Health, Union, NJ) anesthesia, were implanted with osmotic mini-pumps (Alzet model 2001; Alza, Palo Alto, CA) to administer 20 ng/h DAVP, the V2-receptor-selective agonist of vasopressin (n = 6) or saline vehicle (n = 6) for 7-days. In the second study, 205-g male Sprague-Dawley rats were water restricted for 7 days by offering a limited amount of water as a part of a slurry diet (23). "Restricted" rats (n = 6) were given 15 ml water · 200 g body wt⁻¹ · day⁻¹ mixed with 15 g of powdered food. Control rats (n = 6) were given 37 ml water · 200 g body wt⁻¹ · day⁻¹ mixed with 15 g of powdered food. Rats in both groups consumed all of their diet daily and maintained weight throughout the 7-day period. In the third study, 12 male Brattleboro rats (250 g) were given a single acute intramuscular injection of 2 nmol of dDAVP dissolved in saline (n = 6) or saline alone (vehicle) (n = 6) and euthanized after 1 h. The fourth study was the same as the third study, but the rats were euthanized after only 30 min. In all studies, rats were euthanized by decapitation, and both kidneys were rapidly removed and either frozen on dry ice for later processing or immediately dissected and homogenized in a buffered isolation solution as described below.

**Western Blotting**

Immediately after euthanasia (or after thawing), kidneys were placed in chilled-buffered isolation solution containing 250 mM sucrose, 10 mM triethanolamine (Calbiochem, La Jolla, CA), 1 μg/ml leupeptin (Bachem, Torrance, CA), and 0.1 mg/ml phenylmethylsulfonyl fluoride (US Biochemical, Toledo, OH) adjusted to pH 7.6. Whole right kidneys were homogenized using a tissue homogenizer (Omni 2000; Omni International, Warrenton, VA) fitted with a 10-mm micro-sawtooth generator in 10 ml isolation solution on ice. The left kidney was dissected into cortex and inner stripe of the outer medulla. Each region was separately homogenized in either 10 ml (cortex) or 1 ml (outer medulla) of isolation buffer while on ice.

Protein concentrations of the homogenates were measured by the Pierce BCA Protein Assay Reagent Kit (Pierce, Rockford, IL). All samples were then diluted with isolation solution to a protein concentration of between 1 and 3 μg/ml and solubilized at 60°C for 15 min in Laemmli sample buffer. Samples were stored at −80°C until ready to run on gels.

**Electrophoresis and blotting of membranes.** Initially, “loading gels” were done on each sample set. Five micrograms of protein from each sample was loaded into an individual lane, and electrophoresed on 12% polyacrylamide gels (precast; Bio-Rad, Hercules, CA), and then stained with Coomassie blue dye (G-250, Bio-Rad; 0.04% solution made in 3.5% perchloric acid). Gels were then destained with water, and selected bands were scanned (Scan Jet 6100C; Hewlett-Packard, Palo Alto, CA) to determine density (NIH-Image software) and relative amounts of protein loaded in each lane. Finally, protein concentrations were “corrected” to reflect these measurements.

For immunoblotting, 10–30 μg of protein from each sample were loaded into individual lanes of precast minigels of 7, 10, or 12% polyacrylamide (Bio-Rad). The proteins were transferred from the gels electrophoretically to pure nitrocellulose membranes (Bio-Rad). After a 30-min, 5% milk block, membranes were probed overnight at 4°C with the desired affinity-purified polyclonal antibody. The production, purification, and characterization of the α- (L766), β- (L558), and γ- (L550) ENaC antibodies has been previously described in detail (28). Our anti-aquaporin-2 antibody (L141) was made to the same peptide as our previously used L127 antibody (10, 13, 35, 36, 49) and gives a similar labeling pattern. Likewise, our anti-NHE3 antibody (L546) (18) and our anti-NCC antibody (L573) (24) have been previously characterized. For probing blots, all antibodies were diluted into a solution containing 150 mM NaCl, 50 mM sodium phosphate, 10 mg/dl sodium azide, 50 mg/dl Tween 20, and 0.1 g/dl bovine serum albumin (pH 7.5). The secondary antibody was goat anti-rabbit IgG conjugated to horseradish peroxidase (Kirkegaard and Perry Laboratories, Gaithersburg, MD) used at a concentration of 0.1 μg/ml. Sites of antibody-antigen reaction were visualized using luminol-based enhanced chemiluminescence (LumiGLO; Kirkegaard and Perry Laboratories) before exposure to X-ray film (Fujifilm; Fugi Medical Supplies, Stamford, CT).

**Statistics**

Relative intensities of the resulting immunoblot band densities were determined by laser scanning (Scanjet 6100C) followed by analysis with NIH IMAGE software. The statistical significance of the effects of the various treatments on expression was determined by an unpaired t-test of density values when standard deviations were equivalent, or by Welch’s t-test when standard deviations were significantly different (Systat software; Systat, Point Richmond, CA). P < 0.05 was considered statistically significant.

**RESULTS**

**Chronic dDAVP Infusion or Water Restriction Increases Aquaporin-2 Abundance**

As a positive control, aquaporin-2 protein levels were examined in the whole kidney homogenates in the Brattleboro rats from the chronic dDAVP infusion...
examined to assess equivalency of loading. Densitometry of the nonglycosylated form of aquaporin-2 (the 29-kDa band) and the glycosylated form of aquaporin-2 (the bands that fall between ~36 and 47 kDa) was performed, and the values were summed. As we predicted, band density for aquaporin-2 was significantly increased (to 463% of the vehicle level) in the dDAVP-infused rats. Furthermore, water restriction of Sprague-Dawley rats also significantly increased band density for aquaporin-2 to 201% of the level observed in their control (water-replete) animals (Fig. 1B).

**Chronic dDAVP Infusion Increases α-, β-, and γ-ENaC Abundance**

Figure 2, A–C, shows immunoblotting data derived from whole kidney homogenates for the three ENaC subunits in the Brattleboro rats that were either infused with vehicle or dDAVP for 7 days. Figure 2D shows densitometric quantification of the immunoblots. As above, each lane was loaded with a sample from a different rat. In response to dDAVP infusion, β- and γ-ENaC were substantially increased (Fig. 2, B–D), whereas α-ENaC showed a more moderate, yet still significant, increase (Fig. 2, A and D). Average band density of α-ENaC in the dDAVP-infused rats was increased to 142% of the vehicle mean, whereas the average band densities for β- and γ-ENaC were increased to 238% and 288% of the vehicle mean, respectively (Fig. 2D).

**Water Restriction Increases β- and γ-ENaC Abundance**

Water restriction to Sprague-Dawley rats causes a rise in endogenous levels of circulating vaspressin. Figure 3, A–C, shows immunoblots of whole kidney homogenates prepared from Brattleboro rats infused with vehicle (saline) (n = 6) or dDAVP (20 ng/h) (n = 6) for 7 days. Each lane is loaded with a sample from a different rat. Preliminary Coomassie-stained gels were examined in order to assess equivalency of loading. Densitometry of the nonglycosylated form of aquaporin-2 (the 29-kDa band) and the glycosylated form of aquaporin-2 (the bands that fall between ~36 and 47 kDa) was performed, and the values were summed. As we predicted, band density for aquaporin-2 was significantly increased (to 463% of the vehicle level) in the dDAVP-infused rats. Furthermore, water restriction of Sprague-Dawley rats also significantly increased band density for aquaporin-2 to 201% of the level observed in their control (water-replete) animals (Fig. 1B).

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A similar pattern of expression was observed as seen from a different rat. In response to water restriction, a blots. As above, each lane was loaded with a sample shows densitometric quantification of the immuno-body wt $^2$-ENaC were smaller. In Fig. 3 $^2$-ENaC antibody (L558). Similar to the dDAVP infusion, although the increases in $\beta$- and $\gamma$-ENaC were smaller. In Fig. 3A, the immunoblot was probed with polyclonal anti-$\alpha$-ENaC antibody (L766). Although $\alpha$-ENaC tended to be increased, band density was not significantly affected by water restriction. Average band density of $\alpha$-ENaC in the water-restricted rats was 122 $^6$ of the control mean ($P = 0.18$) (Fig. 3B). In Fig. 3 shows an immunoblot loaded with whole kidney homogenates and probed with polyclonal anti- $\beta$- and $\gamma$-ENaC abundance was increased after 30 min, $^5$100%). In the cortex, both $\alpha$- and $\gamma$-ENaC abundances were increased after 30 min, 60 min (Fig. 5A). In contrast, in the outer medulla no significant effects of dDAVP were observed after 60 min (Fig. 5A). In contrast, in the outer medulla no significant effects of dDAVP were observed in this short time frame (Fig. 5B).

Chronic dDAVP Infusion, but not Water Restriction, Increases the Abundance of the Thiazide-Sensitive NaCl Cotransporter

We also assessed the effects of chronically elevated vasopressin levels on two additional sodium transporters: NCC, the thiazide-sensitive, apically located NaCl cotransporter found in the distal convoluted tubule; and NHE3, the sodium/hydrogen exchanger (type III) found in proximal tubules and the thick ascending limb. Figure 4A shows an immunoblot of whole kidney homogenates from the dDAVP-infused Brattleboro rats and their vehicle controls probed with our anti-NCC antibody (L573) (24). NCC abundance was increased significantly (band density was 241% of vehicle mean) by the dDAVP infusion. Figure 4B shows an immunoblot of whole kidney homogenates from control (water-replete) Sprague-Dawley rats and water-restricted rats. With this treatment, there was no change in NCC abundance (mean band density for water-restricted group = 93% of control mean). Figure 4, C and D, shows similar blots that were probed with our anti-NHE3 antibody (L546) (16). NHE3 abundance was not affected in either study.

Acute Exposure to dDAVP Results in Increased Abundance of Cortical ENaC Subunits

In studies designed to examine acute (30–60 min) effects of vasopressin on ENaC abundance, modest, yet significant, increases in ENaC subunit abundances were observed. However, this rapid response was only observed in the cortex homogenates. Figure 5 shows a bar graph summarizing the changes in $\alpha$, $\beta$, and $\gamma$-ENaC expression in the cortex (Fig. 5A) and the outer medulla (Fig. 5B) of the dDAVP-treated Brattleboro rats after 30 or 60 min of exposure to dDAVP (values have been normalized to their vehicle controls = 100%). In the cortex, both $\alpha$- and $\beta$-ENaC abundances were significantly increased after 30 min, and $\beta$- and $\gamma$-ENaC abundances were increased after 60 min (Fig. 5A). In contrast, in the outer medulla no significant effects of dDAVP were observed in this short time frame (Fig. 5B).
DISCUSSION

Vasopressin has been shown by several investigators (9, 18, 20, 40, 41, 45, 51) to acutely increase sodium transport in the CCD of rats and/or rabbits. This transport has been shown to be amiloride sensitive in many of these experiments, suggesting enhanced transport through the apically located amiloride-sensitive ENaC, which is the principal port of sodium entry into these cells (2). With recent cloning of the three subunits of ENaC, α, β, and γ, from the rat colon (7, 9), we are now able to study potential molecular mechanisms responsible for ENaC regulation. Nevertheless, it is important to note that in contrast to the above acute studies, the regulation of sodium transport in the collecting duct by chronically elevated blood vasopressin levels has not yet been adequately studied. It may well be that, like many other proteins, e.g., aquaporin-2, chronic and acute regulation are accomplished by different mechanisms (10, 22, 35, 36). In these studies, we examined the effects of elevated blood vasopressin levels on ENaC subunit abundance after both chronic (7-day infusion of dDAVP, or water restriction) or acute (single injection of dDAVP) exposure. Our principal finding was that all three ENaC subunits, α, β, and γ, are increased by vasopressin, with the increases in β and γ being most pronounced. We show increased whole kidney abundance of α-, β-, and γ-ENaC after a 7-day infusion of dDAVP to Brattleboro rats and an increase in whole kidney β and γ abundance after 7-day water restriction to Sprague-Dawley rats. Furthermore, in Brattleboro rats exposed acutely to dDAVP, increased abundances of cortical α- and β-ENaC at 30 min and cortical β- and γ-ENaC at 60 min were apparent. In contrast, the abundances of ENaC subunits in the outer medullary collecting duct were more resistant to change in response to dDAVP exposure; no changes were apparent in the acute studies, and only β-ENaC was increased after chronic dDAVP.
**Chronic Vasopressin Increases α-, β-, and γ-ENaC Abundance In Vivo**

Our findings are consistent with those of Djelidi et al. (11), who recently reported vasopressin-stimulated increases in β and γ transcription rates and ENaC activity in the rat cortical collecting duct cell line, RCCD1. In the studies of Djelidi et al. (11) in vitro incubation of the cells with vasopressin resulted in a rapid and sustained (up to 10 h) increase in amiloride-sensitive short-circuit current and $^{22}$Na transport. The RNase protection assay showed a rapid (within 1–3 h) increase in γ-ENaC mRNA, with no change in α-ENaC mRNA. Furthermore, in situ hybridization showed an increase in β-ENaC mRNA within 24 h. Treatment with actinomycin D blocked these changes in mRNA, suggesting that increased transcription was responsible for the changes in β and γ rather than decreased degradation of the mRNA. These studies also showed an increase in the rate of protein synthesis of both β- and γ-ENaC, but not α-ENaC, as assessed by immunoprecipitation of $^{35}$S-labeled proteins. When transcription was blocked by actinomycin D, the vasopressin-induced increase in short-circuit current was no longer apparent, suggesting a potentially important role for changes in ENaC subunit abundance to regulate sodium transport capacity of the cells.

Our results are similar in that β- and γ-ENaC expression is increased, but somewhat in contrast with the above studies, we observed an increase in α-ENaC abundance after 30 min and 7 days of dDAVP infusion. These differences could be a result of α-ENaC being increased by a mechanism other than transcriptional or translational regulation in our studies. For example, the rate of degradation of α-ENaC protein might be retarded with high circulating vasopressin levels. Alternatively, it is possible that chronic administration of dDAVP to Brattleboro rats corrected the relative aldosterone deficiency that is normally apparent in untreated Brattleboro rats (4, 31, 32). Although Brattleboro rats have normal or elevated plasma renin activity and angiotensin II (ANG II) levels, their aldosterone levels are approximately twofold lower than their parent strain, the Long-Evans rat (4, 31, 32). The defect in the Brattleboro rat has been proposed to be due to several possible mechanisms. First, these rats may have a decreased secretion of adrenocorticotropic hormone (ACTH) from the pituitary (5). In vitro studies have shown that dDAVP is also a potent $V_{1B}$ vasopressin receptor agonist (43), and Sakai et al. (44) revealed that dDAVP directly stimulates ACTH release from corticotropic adenoma cells through $V_{1B}$ vasopressin receptors. Second, the defect could be at the level of the adrenal, i.e., depressed adrenal responsiveness as result of decreased ANG II (46) or ACTH receptors on the adrenal gland or post-receptor-mediated events (4, 27). Thus the increase in α-ENaC expression after chronic dDAVP might, in part, result from increased circulating aldosterone, as it has been reported by several investigators that α-ENaC mRNA (1, 15, 37) or protein abundance (28) is markedly increased by increases in circulating aldosterone levels. In support of this mechanism, we also found that expression of whole kidney NCC, the NaCl cotransporter of the distal convoluted tubule, was significantly increased by the dDAVP infusion, relative to vehicle infusion (Fig. 4) even though the distal convoluted tubule is believed to lack V$_2$ receptors. NCC abundance has recently been reported (24) to be increased by high circulating levels of aldosterone in Sprague-Dawley rats either infused with aldosterone or fed a low-salt diet. Finally, we observed that, like α-ENaC, NCC abundance was not upregulated by water restriction. Therefore, it is possible that α-ENaC and NCC may have been increased with chronic dDAVP infusion due to a vasopressin-mediated restoration of normal circulating aldosterone levels. Nevertheless, increased aldosterone levels would not likely explain our observation of increased cortical α-ENaC abundance after only 30-min exposure to dDAVP. Furthermore, it is possible that NCC abundance is regulated differently in response to elevated vasopressin in Brattleboro rats (the strain in which the dDAVP-infusion studies were performed) than in Sprague-Dawley rats (the strain in which the water restriction studies were performed). Additional studies will need to be done to sort out specific mechanisms.

**Is α-ENaC Abundance Rate Limiting for Sodium Transport?**

Independent regulation of the three subunits of ENaC is apparent from this work and others (1, 15, 28, 37, 47). The implication of changes in abundance of one or two subunits and not the other(s) on sodium transport capacity is not known. α-ENaC appears to be the only subunit of the three that has been reported to be transcriptionally regulated by aldosterone (1, 15, 37, 47), a hormone which clearly upregulates sodium reabsorption in the collecting duct. May et al. (30) have postulated that α-ENaC abundance is rate limiting for assembly of the multimeric ENaC complex; thus sodium transport might be expected to be proportional to α-ENaC abundance. Therefore, increased β- and γ-ENaC abundance (as we have observed here) with little concurrent change in α-ENaC abundance might be predicted to have little impact on net NaCl absorption. However, the physiological benefit of having increased β- and γ-ENaC expression might become significant, in so far as NaCl reabsorption is concerned, if α-ENaC is upregulated and no longer rate limiting, for instance, by high aldosterone levels. This might occur, for example, during volume depletion, when vasopressin and aldosterone would be predicted to work synergistically to increase NaCl reabsorption. Further studies will be required to assess the impact of differential regulation and subunit stoichiometry on sodium transport.
Potential Role in Acute Upregulation of Sodium Transport

The rapid time frame in which increases in abundance of ENaC subunits occur (30–60 min) would suggest that these abundance changes might be an important component of the acute stimulation of sodium transport in perfused CCDs when vasopressin is added to the bath. Reif et al. (40, 41), Tomita et al. (51), Frindt and Burg (18), and others have observed increased sodium transport that occurs within 20 min, in perfused tubules from rats and/or rabbits. The fact that vasopressin increases β- and α-ENaC abundance (and possibly also γ-ENaC) in the 30-min time frame, might partly explain why vasopressin’s action to increase sodium transport appears to be additive to that of mineralocorticoids (20, 41), as their mechanisms for activating ENaC may be distinct. However, the relative magnitude of the increase in transport observed when vasopressin is applied to the bath in perfused tubule studies (about 3- to 4-fold) would suggest that mechanisms in addition to increased subunit abundance most likely are responsible for increased sodium absorption in the CCD. For instance, trafficking or phosphorylation of any of the ENaC subunits may be an important component of regulation of ENaC activity. Protein kinase A has been reported to increase patch-clamp current in A6 cells (39), and Kleyman et al. (26) have evidence suggesting that ENaC is redistributed to the apical plasma membrane with arginine vasopressin stimulation in A6 cells.

Cortical Collecting Duct Appears Most Sensitive to Vasopressin

Another interesting finding from our acute studies was that the vasopressin-mediated changes in ENaC abundance were mainly, and almost exclusively, observed in the cortical homogenates (CCD) compared with the outer medullary homogenates (outer medullary collecting duct). The reason for this striking difference is unclear. The interstitial environment of the medulla is quite different from that in the cortex, particularly with regard to the increase in osmolality. Furthermore, different paracrine factors may be present, such as prostaglandins. Additionally, although vasopressin V₂ receptors have clearly been localized to all regions of the collecting duct (17, 25, 38, 48), the number of active binding sites or post-receptor intracellular signaling may be different along the length of the collecting duct.

In conclusion, we find a marked increase in whole kidney abundances of β- and γ-ENaC with chronic exposure to vasopressin in rats. Furthermore, α-ENaC abundance is also increased, but much more modestly, and only after dDAVP infusion. Increased subunit abundances might be expected to result in increased amiloride-sensitive sodium transport capacity of the collecting duct. In addition, we find rapid (within 30–60 min) increases in abundances of all three subunits in response to vasopressin. These changes could be predicted to play some role in the increased transport of sodium observed in perfused CCDs when vasopressin is added to the bath, although additional mechanisms are likely to be involved. These combined results therefore suggest that vasopressin is involved in both salt and water conservation, through regulation of ENaC abundance, during water-deprived states, or during states of acutely elevated blood vasopressin, such as hemorrhage.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants 1R01-DK-02672-01 to Georgetown University (to C.A. Ecelbarger and J.G. Verbalis) and by the intramural budget of the National Heart, Lung, and Blood Institute (to C. A. Ecelbarger, G-H. Kim, J. Terris, S. Masilamani, C. Mitchell, I. Reyes, and M. A. Knepper).

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