Vasopressin-elicited water and urea permeabilities are altered in IMCD in hypercalcemic rats

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Vasopressin-elicited water and urea permeabilities are altered in IMCD in hypercalcemic rats. Am. J. Physiol. 274 (Renal Physiol. 43): F978–F985, 1998.—To investigate how hypercalcemia blunts renal concentrating ability, alterations in basal and arginine vasopressin (AVP)-elicited osmotic water (P(w)) and urea (P(urea)) permeabilities were measured in isolated perfused terminal inner medullary collecting ducts (IMCD) from control and chronically hypercalcemic rats after dihydrotachysterol (DHT) (M. Levi, L. Peterson, and T. Berl. Kidney Int. 23: 489–497, 1983) treatment. The IMCD P(w) of DHT-treated rats did not increase significantly after AVP and was accompanied by a significant 87 ± 4% reduction in aquaporin-2 (AQP-2) protein content but not mRNA. In contrast, both basal and AVP-elicited IMCD P(urea) from DHT rats were significantly increased and accompanied by a significant 41 ± 11% increase in AVP-regulated urea transporter protein content. Immunoblotting with anti-calcium/polyvalent cation-sensing receptor protein (CaR) antiserum revealed specific alterations in CaR bands in the presence of an extracellular calcium polyvalent complex changes occurring in the kidney during hypercalcemia. Moreover, we have recently reported many investigators have now shown that the IMCD content of AVP-elicited apical membrane transporter proteins for water (aquaporin-2, or AQP-2) and urea (vasopressin-regulated urea transporter, or VRUT) are both modulated by various physiological stimuli. These include a significant increase in the IMCD content of AQP-2 protein after intervals of prolonged antidiuresis (33), congestive heart failure (28, 43), and chronic AVP administration (11). In contrast, the IMCD urea permeability (P(urea)) and VRUT mRNA are increased (33), whereas IMCD P(w) and AQP-2 protein (33) are reduced after moderate protein restriction. However, potential alterations in the IMCD content of AQP-2 or VRUT protein contents, together with changes in transepithelial IMCD P(w) and P(urea) have not been examined in hypercalcemia. Moreover, we have recently reported the presence of an extracellular calcium polyvalent cation-sensing receptor (CaR) protein in the apical membrane of both rat and human IMCD (32). Acute increases in luminal calcium from 1 to 5 mM in isolated perfused rat terminal IMCD cause a significant, rapid, and reversible 30% reduction in AVP-elicited P(w). These data suggest that the IMCD apical CaR may also contribute to the alterations in IMCD AVP-elicited P(w) present in chronic hypercalcemia. To investigate alterations in IMCD transepithelial transport during sustained hypercalcemia, we have quantified changes in IMCD P(w) and P(urea) as well as determined alterations in renal AQP-2, VRUT, and CaR expression in DHT-treated rats compared with control rats.

METHODS

Animals. All studies were performed on 250-g male Sprague-Dawley adult rats where body weights were obtained on individual rats on days 1 and 14 of the experimental protocol. As reported previously by Levi et al. (21), animals were fed for up to 14 days with a standard commercial rat chow (Prolab

hypercalcemia; vasopressin; kidney; epithelia; transport
Animal Diet RM 3000; PMI Feeds, St. Louis, MO) and given free access to water. The control group was pair fed with the DHT-treated group that received 4.25 mg·kg diet 1·day 1·day of DHT (Roxane Laboratories, Columbus, OH). After induction of anesthesia with intraperitoneal injection of pentobarbital sodium, blood was obtained in selected rats through direct cardiac puncture and analyzed for serum electrolytes (Na, K, Cl), blood urea nitrogen (BUN), calcium, phosphorus, and glucose (Tufts Veterinary Diagnostic Laboratory, North Grafton, MA). Urine osmolality was also measured (Wescor 5100 C vapor pressure osmometer; Wescor, Logan, UT) from the same animals after bladder puncture. The kidneys were harvested, cortex and inner medulla were separated, and samples were prepared, as described below. 

Tissue preparation for tubule micropерfusion. Twenty minutes before each experiment, furosemide (5 mg ip) was administered to reduce medullary osmolality and prevent osmotic shock to the inner medulla after it was removed from the animal and placed into dissecting solution (described below) (31). Initial or terminal IMCDs were dissected as described previously (25, 34) in a dissecting solution gassed with 95% O2-5% CO2 and containing (in mM) 118 NaCl, 25 NaHCO3, 2 CaCl2, 2.5 K2HPO4, 1.2 MgSO4, 5.5 glucose, and 4 creatinine. The tubules were perfused, using standard techniques, in a 37°C bath, which was exchanged continuously with 95% O2-5% CO2 gas (1, 25, 31, 32, 34). 

Osmotic water permeability measurement. To determine P0, creatinine was used as a volume marker (1, 32). Creatinine concentration in perfusate, bath, and collected fluid was measured, using a continuous-flow ultramicrofluorometer as described (32, 34). P0 was measured by increasing the bath osmolality to 490 mosmol/kgH2O by adding NaCl (32, 34). The perfusion rate (V0) was calculated as V0 = V(Cr/Cro), where Cr is the creatinine concentration in the perfusate, Cro is the creatinine concentration in the collected fluid, and V is the collected perfusion fluid. Fluid flux (J0) was calculated as J0 = V0 − V0; P0 was calculated using the equation of Al-Zahid et al. (1), as described previously (34). 

After three to four control collections, 100 µM AVP (Sigma, St. Louis, MO) was added to the bath. After 30 min (27, 32, 33), a second set of three collections with a stable P0 value was obtained to assess the response to AVP. Next, 10 nM AVP was added to the bath, and a third set of three collections with a stable P0 value was obtained. 

Urea permeability measurement. To determine Purea, 5 mM urea was added to the bath solution, and 5 mM raffinose was added to the perfusate to create a 5 mM bath-to-lumen urea gradient without any osmotic gradient (25, 31, 32, 34). Previous studies have shown that the same Purea value is obtained regardless of whether a bath-to-lumen or lumen-to-bath urea gradient is imposed (31). Bath and perfusate solutions were otherwise identical to the dissecting solution as described above. First, basal Purea was measured. Next, 10 nM AVP was added to the bath, and, after 30 min, the response to AVP was measured (25, 34). The urea concentration in perfusate, bath, and collected fluid was measured, using a continuous-flow ultramicrofluorometer, and urea flux, as well as Purea, was calculated, as described previously (25, 31, 32, 34). 

RNA isolation and Northern analyses. Pooled specimens of kidney cortex or inner medulla were prepared from either control or DHT-treated rats. After isolation of total RNA by guanidinium thiocyanate-acid phenol extraction (Teletest B, Friendswood, TX), poly(A)+ RNA was prepared as described previously (32). Aliquots of poly(A)+ RNA were then fractionated by denaturing agarose gel electrophoresis (5 µg/lane), and the mRNA was transferred to nylon membranes (Duralon-UV; Stratagene, La Jolla, CA), ultraviolet crosslinked, and probed sequentially with 32P-labeled cDNAs of rat kidney CaR (30), AQP-2 (15), and, finally, β-actin (NE Blot Kit; New England Biolabs, Beverly, MA). All hybridizations were performed at 55°C (32), whereupon membranes were washed in 0.5× standard sodium citrate/0.1% SDS at either 55°C (RakCaR and β-actin) or 65°C (AQP-2). After autoradiography of membranes at −70°C (32), individual transcripts were quantified by scanning densitometry (NIH Image; National Institutes of Health, Bethesda, MD). RakCaR and AQP-2 transcripts were then normalized to the β-actin content of individual lanes. 

Immunohistochemistry. As described previously (32), rats were perfusion fixed, using freshly prepared 4% paraformaldehyde. Tissue samples were then embedded in OCT compound (Miles, Elkart, IN), snap frozen in 2-methylbutane liquid N2, and stored at −70°C until further use. Immunohistochemistry was performed, as described previously (32), using primary antibodies including affinity-purified anti-AQP-2 antisera (1,100 cortex, 1,25,000 inner medulla) (26) and anti-CaR (1,100-1,500) (32). After blocking and incubation with a primary antiserum listed above, 4-µm sections were washed, incubated with a peroxidase-conjugated donkey anti-rabbit secondary antibody (Jackson ImmunoResearch, Westgove, PA), then developed using immunoperoxidase/aminoperoxidase-carbazole technique, and counterstained with Gill no. 3 hematoxylin solution (Sigma) or methyl green (Fisher, Pittsburgh, PA). Specimens were examined by light microscopy and photographed using a Nikon microscope and camera. Representative images were then scanned and printed. 

Immunoblot analyses. For studies of AQP-2, inner medulla were dissected and protein homogenates were prepared in buffer containing multiple protease inhibitors, as described previously (25, 32, 33). Immunoblotting studies were also performed on purified apical membrane endosomes isolated from control and DHT-treated rats (34). Endosomal proteins (20 µg) were incubated on ice for 30 min in solubilization buffer (8.3 mM Tris, pH 7.4, 125 mM NaCl, 1.0% (wt/vol) Triton X-100, and 1.25 mM pepstatin, 4-µM leupeptin, and 4.8 µM phenylmethylsulfonyl fluoride (final concentrations)) and centrifuged at 100,000 g for 30 min, and the supernatant containing Triton X-100-soluble proteins was mixed with fivefold concentrated Laemelli buffer. These samples were then subjected to SDS-PAGE without exposure to 100°C. For studies on VRUT, inner medulla were dissected into two regions: 1) base and 2) tip, corresponding to the location of the initial and terminal IMCD, respectively, as previously described (3, 25, 32). Tissue dissected from both kidneys of a single rat was placed into ice-cold isolation buffer and processed as described previously (33). SDS-PAGE gel lanes were loaded with 15 µg/lane for VRUT blots probed with anti-VRUT antisera (25) and 50 µg/lane for immunoblots to quantify AQP-2 and CaR proteins. After electrophoresis, proteins were either transferred to polyvinylidene difluoride (PVDF) (VRUT and CaR) or nitrocellulose (AQP-2) and each was processed, as described previously (25, 36, 32, 33). Previous work (19, 25, 40) has verified that quantification of AQP-2 or VRUT contents of individual lanes of immunoblots is carried out under conditions where enhanced chemiluminescence (ECL) signals derived from individual bands are within the linear range. 

Laser densitometry was used to quantify VRUT, CaR, and AQP-2 bands. Data are expressed as arbitrary units per milligram of protein loaded. In all cases, parallel gels stained with either Coomassie blue (PVDF) or Ponceau S (nitrocellulose) showed uniformity of loading.
Statistics. Data are presented as means ± SE (n), where n is the number of rats studied. Statistical significance was considered at P < 0.05. For the perfused tubule experiments, data from three to four collections were averaged to obtain a single value for each experimental phase in each tubule. To test for statistically significant differences, an analysis of variance was used, followed by a multiple comparison, protected t-test (37).

RESULTS

DHT-induced alterations in serum, urine, and body weight parameters. As reported previously by Levi et al. (21) and Peterson (29), who studied rats for intervals of 3 and 7 days, respectively, pair-fed rats receiving 4.25 mg·kg diet⁻¹·day⁻¹ of DHT gained significantly (P < 0.05, n = 22) less body weight (in kg) over the 14-day interval (0.29 ± 0.004), compared with matched controls (0.36 ± 0.007). On day 14, analyses of their serum revealed that the DHT-treated rats exhibited significant increases in calcium (12.6 ± 0.1 vs. 9.6 ± 0.1 mg/dl), sodium (137.4 ± 1.0 vs. 133.5 ± 1.6 meq/l), glucose (222.6 ± 4.6 vs. 204.6 ± 4.6 mg/dl), and osmolality (288 ± 1.6 vs. 280.1 ± 3.0 mosmol·kg⁻¹·H₂O). No significant differences were observed in serum potassium (5.3 ± 0.2 vs. 5.5 ± 0.1 meq/l), chloride (98.7 ± 0.9 vs. 97.2 ± 0.8 meq/l), or BUN (137.4 ± 0.8 vs. 159.5 ± 0.5 mg/dl). DHT-treated rats exhibited a significant reduction of ~28% in urine osmolality compared with controls (771 ± 6.0 vs. 1,067 ± 8.0 mosmol·kg⁻¹·H₂O).

DHT-induced hypercalcemia selectively decreases AVP-elicted Pᵣ in DHT-treated rats. In terminal IMCDs from control rats, basal Pᵣ was 138 ± 31 µm/s (n = 6, Fig. 1). Pᵣ was increased significantly following the addition of 100 nM AVP to the bath (571 ± 123 µm/s) and increased further by 10 nM AVP (836 ± 169 µm/s, P < 0.01). The basal Pᵣ measured in terminal IMCDs from DHT-treated rats (252 ± 33 µm/s, n = 6) was not significantly different from control rats. However, unlike IMCDs from control rats, there was no significant increase observed in Pᵣ in response to either 100 pM AVP (303 ± 52 µm/s) or 10 nM AVP (341 ± 77 µm/s) in terminal IMCDs from DHT-treated rats.

In contrast, basal Pᵣ in terminal IMCDs from DHT-treated rats (91 ± 10 ± 10⁻⁵ cm/s; n = 5; Fig. 2, right) compared with that from control rats (48 ± 8 ± 10⁻⁵ cm/s; n = 5). Moreover, AVP significantly increased Pᵣ in terminal IMCDs from both DHT-treated rats (139 ± 7 ± 10⁻⁵ cm/s) and control rats (101 ± 7 ± 10⁻⁵ cm/s). The value for AVP-elicted Pᵣ in the terminal IMCD of DHT-treated rats was ~38% larger compared with that for controls.

There was no significant difference in basal Pᵣ in initial IMCDs between control rats (6 ± 2 ± 10⁻⁵ cm/s, n = 5; Fig. 2, left) and that of DHT-treated rats (8 ± 1 ± 10⁻⁵ cm/s, n = 5). AVP (10 nM) had no significant effect on Pᵣ in initial IMCDs from control rats (8 ± 2 ± 10⁻⁵ cm/s) but significantly increased Pᵣ in initial IMCDs from DHT-treated rats (17 ± 2 ± 10⁻⁵ cm/s).

Inner medulla of DHT-treated rats exhibits a significant reduction in its content of AQP-2 protein while its content of VRUT protein is significantly increased. Alterations in immunoreactive AQP-2 and VRUT protein were assessed using quantitative immunoblotting of homogenates derived from the inner medulla of both control and DHT-treated rats. As shown in Fig. 3, both the 28-kDa (P < 0.05) and the broad 35- to 45-kDa band (P < 0.02) of AQP-2 in inner medulla from DHT-treated rats were significantly reduced by ~87 ± 4% (n = 6) compared with controls. This decrease in AQP-2 protein was also apparent by light microscopy examination of immunocytocchemistry sections of inner medulla of DHT-treated and control rats when matched slides were processed in a series of paired experiments (n = 2) (Fig. 4). No alterations in the localization of AQP-2

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**Fig. 1.** Osmotic water permeability (Pₒ) measurements in terminal inner medullary collecting duct (IMCD) cells, obtained from dihydrotestosterone (DHT)-treated and control rats. In terminal IMCDs from control rats, arginine vasopressin (AVP, 100 pM–10 nM added to bath) significantly increased Pₒ. In terminal IMCDs from DHT-treated rats, AVP had no significant effect on Pₒ; n = 6 tubules in each group. *P < 0.01 vs. basal value, †P < 0.01 between control and DHT-treated rats.

**Fig. 2.** Urea permeability (Pᵤ) measurements in initial and terminal IMCDs obtained from DHT-treated and control rats. Left: in initial IMCDs (n = 5), basal Pᵤ was not significantly different in control and DHT-treated rats. AVP (10 nM added to the bath) significantly increased Pᵤ in initial and terminal IMCDs from DHT-treated rats but had no effect on initial IMCDs from control rats. *P < 0.01 vs. basal value. Right: in terminal IMCDs (n = 5), basal Pᵤ is significantly different between control and DHT-treated rats. AVP (10 nM added to the bath) significantly increased Pᵤ in terminal IMCDs from control and DHT-treated rats. *P < 0.01 vs. basal value, †P < 0.01 between control and DHT-treated rats.
protein to the apical membrane region of IMCD cells were observed in DHT-treated rats.

In contrast, immunoreactive VRUT protein, present as a 97-kDa band shown in Fig. 5, was increased by 41 ± 11% in the inner medullary tip of DHT-treated rats compared with controls (n = 5, P < 0.01). However, there was no significant change in VRUT protein in the inner medullary base (Fig. 5; n = 5, P = 0.09).

Northern analyses reveal no significant alterations in steady-state levels of either AQP-2 or CaR in inner medullary mRNA from DHT-treated and control rats. To determine whether alterations in the steady-state level of AQP-2 mRNA occur in DHT-treated rats, blots containing mRNA from kidney cortex and inner medulla were sequentially probed with cDNAs of AQP-2, RaKCaR, and then β-actin. As shown in Fig. 6, no significant differences in the 1.9-kb AQP-2 mRNA content of either kidney cortex (P = 0.07) or inner medulla (P = 0.1) were observed in DHT-treated rats vs. control rats (n = 5).

To assess whether DHT administration alters the steady-state level of the CaR transcripts present in epithelial cells of both the cortical TAL (30) and IMCD (32), the same blots were probed with a 32P-labeled CaR cDNA, as shown in Fig. 6. No significant differences were apparent in either the 7- to 7.5-kb (P = 0.09) or 4-kb (P = 0.2) CaR transcripts present in the inner medulla of DHT-treated rats, compared with control rats (n = 5). In contrast, these same transcripts were both increased significantly by ~75% for the 7- to 7.5-kb transcript (P = 0.002) and 65% for the 4-kb CaR transcript (P = 0.005, n = 5) in the cortex of the DHT-treated rats.

Immunoblots of purified apical membrane endosomes from DHT-treated rats reveal alterations in CaR-immunoreactive protein bands compared with control rats. Recent studies (5, 9, 42) have shown that CaR proteins exist as multiple bands on CaR-specific immunoblots that correspond to monomeric, dimeric, and high-molecular-weight CaR species. The CaR-reactive bands are present in various rat tissues (34), as well as in cells expressing recombinant CaR proteins (5, 8). In each case, CaR bands of 121 kDa, a 138- to 169-kDa doublet, and a 240- to 310-kDa band, corresponding to

![Fig. 3.](image1) Representative immunoblot showing alterations in the aquaporin 2 (AQP-2) protein content of the inner medulla from DHT-treated vs. control rats. Top: representative immunoblot of protein from total cell lysate of inner medulla from control or DHT-treated rats probed with anti-AQP-2 antibody (26). Bottom: identical membrane used for immunoblot, top, stained for protein showing identical protein content. Representative of a total of 6 experiments.

![Fig. 4.](image2) Representative light microscopy immunocytochemistry sections showing an apparent reduction in the AQP-2 content of individual terminal IMCDs in DHT-treated compared with control rats. Immunocytochemistry was performed as described previously (34), where binding of specific anti-AQP-2 antibody is indicated by the rose-colored reaction product. Sections from both DHT-treated and control rats were developed using immunocytochemistry under identical conditions in paired experiments. A: section from the inner medullary tip of control rats (magnification, ×2,500). B: section from inner medullary tip of DHT-treated rats (magnification, ×2,500). Note that individual tubules possess less apparent AQP-2 protein.
nonglycosylated (121 kDa) and glycosylated (138–169 kDa) monomeric and dimeric (240–310 kDa) CaR proteins, respectively, have been reported previously (5, 8, 9, 42).

To determine whether the abundant CaR protein present in endosomes derived from the apical membrane of IMCD (32) are altered after DHT administration, apical membrane endosomes were purified from paired DHT vs. control rats and immunoblotted with a specific anti-CaR antibody. As shown in Fig. 7, CaR bands corresponding to molecular masses of 121 kDa, 138–169 kDa, and 240–310 kDa are present in purified endosomes from control and DHT-treated rats. However, the staining intensity of CaR-reactive bands of 169 and 310 kDa appeared markedly reduced in DHT samples compared with paired controls. Although the functional significance of these observations is un-

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**Fig. 5.** Quantitative immunoblot analyses of the vasoressin-regulated urea transport (VRUT) content of the inner medulla of DHT-treated and control rats. **A:** representative immunoblot of protein from total cell lysate of inner medulla (I.M.) base (left) or tip (right) from control or DHT-treated rats probed with VRUT antibody (25). Each lane shows protein from an individual rat. VRUT antibody detects protein bands at 97 and 117 kDa in the I.M. tip (40) but only a 97-kDa VRUT protein band in the I.M. base. Band at 97 kDa is significantly increased in the I.M. tip from DHT-treated rats compared with control rats. **B:** summary of laser densitometry analysis. The 97-kDa VRUT protein is not significantly different between control and DHT-treated rats. However, the 97-kDa VRUT protein in the I.M. tip from DHT-treated rats is significantly increased compared with control rats; n = 5. *P < 0.01.

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**Fig. 6.** Northern blotting analysis of steady mRNA levels for AQP-2, cation-sensing receptor (CaR), and β-actin in the inner medulla from DHT-treated and control rats. A single filter containing 5 μg of poly(A)⁺ RNA from either the superficial cortex (lanes 2 and 4) or inner medulla/papilla (lanes 1 and 3) of DHT-treated (lanes 1 and 2) or control (lanes 3 and 4) rats were probed sequentially with 32P-labeled cDNAs for rat kidney CaR (A), AQP-2 (B), or β-actin (C). Note that DHT-treated rats exhibit an increase in both 7- and 4-kb transcripts in the superficial cortex but not inner medulla. Arrows denote respective sizes of the various transcripts. DM and CM, DHT-treated and control medulla; DC and CC, DHT-treated and control cortex.

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**Fig. 7.** Purified apical membrane endosomes from IMCD of DHT-treated rats exhibit alterations in CaR-reactive bands compared with controls. In two independent experiments (i and ii), identical quantities of Triton X-100-solubilized and -purified endosomal protein from either control (C) or DHT-treated (DHT) rats were subjected to immunoblotting with anti-CaR antibody in the absence (−) or presence (+) of immunizing peptide. Apparent molecular masses of the CaR-specific species observed are indicated by arrowheads, left. Note that CaR-reactive bands of 169 kDa and 310 kDa (*) are reduced in DHT vs. control endosomes.
known at present, these data suggest that the CaR present in the IMCD apical membrane may be modified in some manner in rats receiving DHT treatment.

**DISCUSSION**

The data displayed in Figs. 1–7 reveal a complex series of alterations in IMCD transepithelial transport in response to DHT-induced hypercalcemia. Figure 1 shows that the normal AVP-elicited increases in P, are virtually ablated in IMCD obtained from DHT-treated rats compared with controls. However, DHT treatment does not significantly alter basal P, in rat IMCD. These data are consistent with previous reports (21, 29) showing that DHT-treated rats exhibited a significant 65% reduction in maximal urinary osmolality compared with controls, despite the fact that DHT-treated rats possessed comparably elevated serum AVP levels.

The reduction in AVP-elicited IMCD P, observed in DHT-treated rats was accompanied by an 87% reduction in AQP-2 protein content in the inner medulla (Fig. 3), without apparent redistribution of AQP-2 protein, as detected by immunocytochemistry (Fig. 4). The significant reduction of AQP-2 in DHT-treated inner medulla likely contributes to the lack of AVP responsiveness by the IMCD. These data are similar to those reported in chronic lithium intoxication (22), bilateral ureteral obstruction (14), hypokalemia (23), nephrotic syndrome (2), and protein restriction (33) in rats where either IMCD P, and/or AQP-2 protein content are reduced. However, DHT-associated reductions of inner medullary AQP-2 protein content were not accompanied by significant reductions in AQP-2 mRNA content, as has been reported for these other causes of reduced urinary concentrating ability. In this regard, it is possible that DHT-induced hypercalcemia may reduce IMCD AQP-2 protein not through transcriptional mechanisms but rather via posttranscriptional mechanisms, including, perhaps, activation of calpain protease activity (41).

In contrast, IMCD P, and inner medullary VRUT content are both increased significantly in DTH-treated rats compared with controls (Figs. 2 and 5). The enhanced inner medullary VRUT content may contribute to the increased basal as well as AVP-elicited IMCD P,. These data may also account for previous reports (21) showing that the significant decrease in medullary solute content present in DHT-treated rats compared with controls is due primarily to a decrease in nonurea solutes (i.e., NaCl) rather than urea. Our data are also consistent with previous studies (6) showing that IMCD adenylyl cyclase is fully functional in IMCD from DHT-treated rats, since AVP-elicited increases in P, are regulated through increases in intracellular cAMP (31, 34). Taken together, these data suggest that IMCD of DHT-treated rats undergo a selective enhancement of AVP-elicited P, and VRUT, while AQP-2-mediated, AVP-elicited P, is significantly reduced via a process activated after the generation of cAMP by the IMCD.

Figures 6 and 7 display data showing that DHT treatment alters CaR mRNA and protein in the kidney compared with controls. Significant increases in both the 7- and 4-kb CaR transcripts present in the renal cortex are observed after DHT treatment (Fig. 6). These data confirm and extend recent studies of Brown et al. (7), who reported that DHT administration to vitamin D-deficient rats produced significant increases in CaR transcripts in whole kidney mRNA. These alterations may contribute to the significant reduction in hormone-stimulated TAL adenyl cyclase activity reported in DHT-treated rats (6), as well as in isolated rat TALs exposed to increases in extracellular calcium concentrations (38, 39). Furthermore, recent data suggest that CaR inhibits cAMP-mediated increases in furosemide-sensitive Na-K-2Cl cotransport, which provide the driving force for TAL Ca" + and Mg" + reabsorption (18).

In contrast to the TAL, no significant alterations in CaR transcripts were observed in the inner medulla from DHT-treated rats compared with controls. These data suggest that expression of IMCD CaR is differentially regulated compared with TAL CaR. As shown in Fig. 7, multiple CaR protein bands are present in purified endosomes derived from the apical membrane of IMCD that correspond to CaR protein species present in cultured HEK cells expressing recombinant CaR protein (5). At present, we cannot assign specific functional consequences to the alterations observed in CaR-reactive protein bands from IMCD. However, we speculate that interactions between the apical CaR and AQP-2 proteins contribute to the reduction in IMCD P, observed in DHT-treated rats (Fig. 1). Data from previous reports (24) demonstrate that rats made acutely hypercalcemic by vitamin D exhibit 10-fold increases in mean concentrations of urinary calcium (89.4 ± 39.3 mg/100 ml) compared with paired controls (8.1 ± 2.2 mg/100 ml). Moreover, recent data from our laboratories demonstrate identical changes in CaR-immunoreactive protein species present in AQP-2 endosomes purified from DHT-treated rats that are accompanied by alterations in the interaction of CaR with Ca" + and other CaR agonists in vitro (42).

The results of the present study provide new insights into renal mechanisms permitting the kidney to dispose of increased filtered calcium loads in chronic hypercalcemia. Taken together with observations from previous studies, these data suggest that the alterations in renal tubular transport present in DHT-treated rats do not constitute a series of derangements in isolated epithelial cells but rather a coordinated response by different nephron segments to integrate important aspects of both divalent mineral and water metabolism. Inhibition of TAL Na-K-2Cl cotransport by the basolateral TAL CaR protein (18) reduces electrogenic Ca" + and Mg" + reabsorption by the TAL and provides less nonurea solute for the medullary counter-current exchange process (21). The resulting hypotonic fluid containing divalent cations, NaCl, and H2O is then presented to the IMCD after traversing the distal convoluted tubule, as well as cortical and outer medullary collecting ducts. Ablation of AVP-elicited water reabsorption in the terminal IMCD reduces development of maximal urinary osmolality by ~30-40% and thus reduces possible formation of calcium-
containing renal stones. Although the exact mechanisms causing the reduction of AVP-elicted Prf are currently unclear, the present data raise the possibility that the combination of a significant reduction in AQP-2 protein, together with activation of a CaR in the IMCD apical membrane that may influence the trafficking of AQP-2 through interactions with apical membrane signaling proteins, may produce alterations in IMCD response to AVP (20).

In this regard, augmentation of both basal and AVP-elicted urea reabsorption in hypercalcaemia may provide a compensatory mechanism to reduce the magnitude of renal medullary washout. These alterations in Prf may also be important for enhanced conservation of body urea stores, since hypercalcaemia suppresses both appetite and intestinal motility, resulting in a reduction in net urea production from intestinal sources (35).

In conclusion, we have reported alterations in the expression of AQP-2, VRUT, and CaR, accompanied by functional changes in Prf and Pu in the IMCD of hypercalcaemic rats. These alterations in IMCD transepithelial transport, along with reductions in TAL NaCl reabsorption, likely serve as protective mechanisms in the kidney during intervals of sustained hypercalcaemia to prevent the formation of calcium containing renal stones.

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REFERENCES


