Vasopressin induces expression of the Cl\(^{-}/\)HCO\(_3\)^{-} exchanger SLC26A7 in kidney medullary collecting ducts of Brattleboro rats

Snezana Petrovic,1,3 Hassane Amlal,1 Xuming Sun,1 Fiona Karet,2 Sharon Barone,1 and Manoocher Soleimani1,3

1Department of Medicine, University of Cincinnati School of Medicine, and 3Research Services, Veterans Affairs Medical Center, Cincinnati, Ohio; and 2Department of Medical Genetics and Division of Renal Medicine, University of Cambridge, Cambridge, United Kingdom

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Am J Physiol Renal Physiol 290: F1194–F1201, 2006. First published December 13, 2005; doi:10.1152/ajprenal.00247.2005.—SLC26A7 is a newly identified anion exchanger specific to α-intercalated cells of the outer medullary collecting duct (OMCD). The purpose of the present experiments was to examine the expression of SLC26A7 in kidneys of vasopressin-deficient Brattleboro rats before and after treatment with desamino-Cys\(^{1},\) α-Arg\(^{8}\)-vasopressin (dDAVP). Brattleboro rats were treated with dDAVP, a vasopressin analog, for 8 days, and their kidneys were examined for the expression of SLC26A7 protein. The expression of SLC26A7 protein, as examined by immunofluorescence, was undetectable in kidneys of Brattleboro rats. However, treatment with dDAVP induced expression of SLC26A7 protein, restoring it to levels observed in normal rats. These results were verified by Western blot analysis. The mRNA expression of SLC26A7 remained unchanged in response to dDAVP. Immunofluorescent labeling demonstrated abundant levels of anion exchanger type 1 in the OMCD of Brattleboro rats and a mild reduction in response to dDAVP. The abundance of H\(^{+}\)-ATPase was not affected by dDAVP. The increased SLC26A7 expression directly correlated with enhanced aquaporin-2 expression, which is proportional to increased interstitial osmolarity in the medulla. In conclusion, vasopressin increases the expression of SLC26A7 protein through posttranscriptional mechanisms in the OMCD. The induction of SLC26A7 by vasopressin in OMCD cells of Brattleboro rats is likely an attempt by cells to regulate their cell volume and maintain HCO\(_3\)^{-} absorption in a state associated with increased interstitial medullary osmolarity.

Address for reprint requests and other correspondence: S. Petrovic or M. Soleimani, Div. of Nephrology and Hypertension, Dept. of Medicine, Univ. of Cincinnati, 231 Albert Sabin Way, MSB 259G, Cincinnati, OH 45267-0585 (e-mail: Snezana.Petrovic@uc.edu or Manoocher.Soleimani@uc.edu).

SLC26A7 is one of the newly cloned members of the SLC26 family (17, 36). Recent studies from our laboratory demonstrated that SLC26A7 can function as a Cl\(^{-}/\)HCO\(_3\)^{-} exchanger and is expressed in the kidney and stomach (5, 22, 24). Expression of SLC26A7 in the stomach is limited to the basolateral membrane of the acid-secreting parietal cells (22). In kidney, SLC26A7 localizes to the basolateral membrane of acid-secreting α-intercalated cells of the outer medullary collecting duct (OMCD) (5, 24), the nephron segment with the highest rate of acid secretion among the collecting duct segments (28, 40). Secretion of acid in α-intercalated cells of the OMCD occurs via the vacuolar H\(^{+}\)-ATPase in conjunction with H\(^{+}\)-K\(^{+}\)-ATPase and results in the generation of intracellular HCO\(_3\)^{-}, which is then transported across the basolateral membrane via the Cl\(^{-}/\)HCO\(_3\)^{-} exchanger (28, 40). Colocalization of SLC26A7 with AE1 on the basolateral membrane of α-intercalated cells suggests an important role for SLC26A7 in acid secretion and HCO\(_3\)^{-} absorption in the OMCD (5, 24). A recent study indicated that SLC26A7 can function as a Cl\(^{-}\) channel that is regulated by intracellular pH in the heterologous expression system (15). The discrepancy between those results and our observations that consistently demonstrate mediation of Cl\(^{-}/\)HCO\(_3\)^{-} exchange by SLC26A7 (22, 24) may, in part, be due to the use of different expression systems, as well as differences in the interpretation of the results. In support of this latter possibility, removal of perfusate Cl\(^{-}\) resulted in cell alkalization in human embryonic kidney (HEK-293) cells (Fig. 6 in Ref. 15), an observation that can be interpreted to

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indicate that SLC26A7 is a Cl⁻/HCO₃⁻ exchanger. Other investigators have also found that expression of human SLC26A7 in Xenopus oocytes increases Cl⁻/HCO₃⁻ exchange activity (S. L. Alper, personal communication).

One unique property of the kidney medulla is its elevated interstitial osmolarity, which serves as a driving force for water reabsorption. Our functional studies in Xenopus oocytes demonstrated that increasing the medium osmolarity significantly stimulated the SLC26A7-mediated Cl⁻/HCO₃⁻ exchanger activity (24), suggesting that SLC26A7 functions more efficiently in a hypertonic environment, at least in vitro. Desamino-Cys¹,D-Arg⁸-vasopressin (dDAVP), a vasopressin analog, restores the interstitial osmolarity of the medulla, upregulates aquaporin (AQP)-2, and normalizes urine output and toxicity in vasopressin-deficient Brattleboro rats without compromising renal function or causing volume depletion (7, 10, 35). We therefore decided to examine the effect of dDAVP treatment on the expression of SLC26A7 in Brattleboro rats.

MATERIALS AND METHODS

Animals. Brattleboro rats (180–210 g body wt; Harlan, Indianapolis, IN) were placed in metabolic cages with free access to tap water and standard rat chow. The animals were divided into two groups and injected subcutaneously with the vasopressin V₂ receptor analog dDAVP or the equivalent amount of physiological saline (diluent) for up to 8 days. Thereafter, the animals were euthanized with an overdose of pentobarbital sodium, their kidneys were harvested, and their blood was collected.

Sprague-Dawley rats (200 g body wt, Harlan) were divided into four groups: a control group, a group given 0.9% saline in their drinking water, a group injected with deoxycorticosterone acetate (DOCA, 200 mg/kg body wt), and a group given 0.9% saline and injected with DOCA. Non-DOCA-treated animals were injected with an equivalent amount of saline (diluent). After 8 days of treatment, the animals were euthanized with an overdose of pentobarbital sodium, and their kidneys harvested. All procedures were approved by the Institutional Animal Care and Use Committee.

RNA isolation and Northern blot hybridization. Total cellular RNA was extracted from rat kidney zones (cortex, outer medulla, and inner medulla) according to established methods (8), quantitated spectrophotometrically, and stored at −80°C. Total RNA samples (30 µg/lane) were fractionated on a 1.2% agarose-formaldehyde gel, transferred to Magna NT nylon membranes, cross-linked by UV light, and baked. Hybridization was performed according to Church and Gilbert (9). The membranes were washed, blotted dry, and exposed to a PhosphorImager screen (Molecular Dynamics, Sunnyvale, CA). A 32P-labeled cDNA fragment corresponding to nucleotides 8–550 of SLC26A7 cDNA (24) was used as a probe for Northern hybridization. For the AQP2 probe, a 296-bp PCR fragment was generated using the following primer: 5'-AGCGCGCAGAAGTCGGAGCA-3' (sense, bases 102–121) and 5'-CAGCCACATAGAAGGCAGCT-3' (antisense, bases 378–397) (2, 3).

A

Apical H⁺-ATPase labeling of intercalated cells of BB rat

B

Cortex

Fig. 1. A: fluorescent images of regions of interest (ROIs), i.e., labeled cell membranes, in kidney outer medulla. B: position and dimensions (0.7 × 0.7 mm) of fields used for analysis. BB, Brattleboro.
Antibodies. A rabbit polyclonal antibody raised against a mouse SLC26A7 synthetic peptide with the amino acid sequence CGAKRRKRSVLWGKMIHTP (using the mouse expressed sequence tag with GenBank accession no. BB666404) was used for Western blotting and immunofluorescence labeling studies (5, 24). AE1 antibodies were purchased from Chemicon International (Temecula, CA) and had been used previously (5). H+-ATPase immune serum is a rabbit polyclonal antibody raised against the α1-subunit of H+-ATPase (32). Na+/H+ exchanger (NHE) type 1 (NHE1) antibodies raised against the entire COOH-terminal end of NHE1 were purchased from Chemicon International.

Immunofluorescence labeling studies. Rats were euthanized with an overdose of pentobarbital sodium and perfused through the left ventricle with 0.9% saline followed by cold 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). The kidneys were removed, cut into tissue blocks, and fixed in formaldehyde solution overnight at 4°C. The tissue was frozen on dry ice, and 6-μm sections were cut with a cryostat and stored at −80°C.

For staining, cryosections were washed twice in 0.01 M PBS (pH 7.4) and blocked with 10% goat serum for 30 min at room temperature. The cells were permeabilized in 0.3% Triton X-100-PBS for 45–60 min. Primary antibodies were diluted 1:40 (SLC26A7), 1:100 (AE1), and 1:400 (H+-ATPase) in 0.3% Triton X-100-PBS and applied to sections overnight at room temperature. The sections treated with the primary antibodies were rinsed twice in 0.01 M PBS for 10 min and then incubated with a secondary antibody for 2 h at room temperature. Alexa Fluor 488 (green) or Alexa Fluor 568 (red) goat anti-rabbit antibody was used as secondary antibody. After they were washed, the slides were incubated with the nuclear dye Hoechst 33342 at 1:10,000 dilution for 20 min. The sections were then washed four times, air-dried, and mounted in Vectashield mounting medium (Vector Laboratories, Burlingame, CA).

The sections were examined on an epifluorescent microscope (Eclipse 600, Nikon Bioscience, Melville, NY) equipped with a Spot digital camera (Diagnostic Instruments). Digital images were acquired using Spot Advanced software, which was provided with the camera. Acquisition parameters were kept constant between the samples that were compared to allow for later comparisons of the intensity of fluorescent labeling. Simple PCI imaging software (Compix, Imaging Systems, Cranberry Township, PA) was used for quantitative image analysis. Regions of interest were determined by application of the intensity threshold. Minimal and maximal intensities were adjusted until the objects of interest, i.e., labeled cell membranes, were selected (Fig. 1A). Separate multiple regions of interest were applied to parts of the slide without specific labeling to calculate the background level. Background intensity was subtracted from the main measurements. The intensity of the labeling (defined as the mean pixel intensity) and the number of selected objects (i.e., number of labeled cells) were calculated. Multiple fields (0.7 × 0.7 mm at ×200 magnification, 5 fields per slide) were analyzed (Fig. 1B). Only slides processed at the same time with the same concentrations of the primary and secondary antibodies applied under the identical protocols were compared.

Immunoblot analysis of SLC26A7. Microsomal membrane proteins were isolated from kidney outer medulla according to established protocols (5). Proteins were resolved by SDS-PAGE (100 μg/lane) and transferred to a nitrocellulose membrane. The membrane was blocked with 5% milk proteins and then incubated for 6 h with antibody against SLC26A7 (5, 24). The secondary antibody was a donkey anti-rabbit IgG conjugated to horseradish peroxidase (Pierce, Rockford, IL). The results were visualized using chemiluminescence (SuperSignal Substrate, Pierce) and captured on light-sensitive imaging film (Kodak, Rochester, NY).

Materials. [32P]dCTP was purchased from New England Nuclear (Boston, MA); microcellulose filters and other chemicals from Sigma Chemical (St. Louis, MO); RadPrime DNA labeling kit from GIBCO BRL; mmessage mMACHINE kit from Ambion (Austin, TX); and Alexa Fluor-conjugated secondary antibodies and Hoechst 33342 from Molecular Probes (Eugene, OR).

Statistical analyses. Values are arithmetic means ± SE. Comparisons were done using unpaired Student’s t-test. P < 0.05 was considered statistically significant. Microsoft Excel, ProStat, and PSI-Plot were used for statistical analysis.

RESULTS

Effect of dDAVP treatment on urine osmolarity, urine output, and blood chemistry. Brattleboro rats were injected subcutaneously for 8 days with the vasopressin V2 receptor analog dDAVP in a step-wise dose-up schedule (from 0.5 to 5 μg) to induce a state of antidiuresis similar to that of normal rats. Urine output decreased from 192 ml/24 h before treatment to 18 ml/24 h after 8 days of treatment with dDAVP (P < 0.001, n = 4). Urine osmolarity increased from 107 mosmol/kgH2O before treatment to 1,125 mosmol/kgH2O after 8 days of treatment with dDAVP (P < 0.01, n = 4). Serum HCO3− decreased from 23 mM before treatment to 20 mM after 8 days of treatment with dDAVP (P < 0.04, n = 4). The drop in serum HCO3− was consistent with the generation of metabolic acidosis, as verified by increased expression of ammoniagenesis pathway enzymes, including glutaminase and phosphoenolpyruvate carboxykinase by dDAVP (Amlal H, Sulaiman S, Faroqui S, Ma L, Barone S, Petrovic S, and Soilemani M, unpublished observations).

Fig. 2. Effect of desamino-Cys1,D-Arg8-vasopressin (dDAVP) on aquaporin-2 (AQP2) mRNA expression. A: Northern blots of AQP2 in outer medulla of dDAVP-treated rats. AQP2 mRNA was upregulated in dDAVP-treated rats. B: densitometric analysis (i.e., ratio of AQP2 mRNA to 28S rRNA) of results in A. Values are means ± SE (n = 4). **P < 0.002.
Effect of dDAVP on AQP2 mRNA expression. AQP2 mRNA was upregulated (~2-fold) in the outer medulla of rats treated with dDAVP (Fig. 2). This finding confirms previously published results (4, 11) and is consistent with increased interstitial osmolarity in the medulla of dDAVP-treated Brattleboro rats.

Effect of dDAVP on expression of SLC26A7 in kidney outer medulla. Expression of SLC26A7 was examined by immunofluorescence labeling in the outer medulla of dDAVP-treated Brattleboro rats. SLC26A7 labeling is almost absent in the outer medulla of Brattleboro rats (Fig. 3A). Examination of numerous fields on multiple slides revealed occasional dim labeling with SLC26A7 antibody. Treatment of Brattleboro rats with dDAVP heavily upregulates the expression of SLC26A7 (Fig. 3, B and C). Quantitative analysis of multiple fields from several animals demonstrated a significant increase in the number of SLC26A7-expressing cells in response to dDAVP treatment (Fig. 3D), with the number of cells increasing from 20 cells per 20 fields per 4 animals in Brattleboro rats to 928 cells per 20 fields per 4 animals in dDAVP-treated rats. The intensity of the cell membrane fluorescent labeling was 59.5 ± 1.7 (n = 928 cells per 20 fields per 4 animals) in the outer medulla of vehicle-treated rats and 124.7 ± 5.1 (n = 20 cells/20 fields/4 animals) in the outer medulla of dDAVP-treated rats (P < 0.0004).

Next we examined the effect of dDAVP on mRNA expression of SLC26A7. The mRNA expression of SLC26A7 did not change in response to dDAVP treatment (Fig. 4A). Densitometric analysis of the Northern hybridization experiments indicated mRNA levels of SLC26A7 of 82 ± 7% and 101 ± 9% in dDAVP- and vehicle-treated Brattleboro rats, respectively (P > 0.05).

The abundance of SLC26A7 was examined by Western blot analysis on microsomal membranes isolated from the outer medulla. SLC26A7 abundance was significantly increased in rats treated with dDAVP (Fig. 4B). Densitometric analysis of the results demonstrated an ~5.2 ± 0.5-fold increase in SLC26A7 expression in rats treated with dDAVP.

An amiloride-sensitive Na⁺/H⁺ exchanger is located on the basolateral membrane of α-intercalated cells in the OMCD (12, 40), which, on the basis of its properties and localization, is likely NHE1. We examined the effect of dDAVP on the expression of NHE1 in microsomal membranes isolated from the outer medulla. The Western blot analysis in Fig. 4C shows very low expression of NHE1 in the outer medulla of vehicle-treated Brattleboro rats but significantly enhanced expression in dDAVP-treated rats. Densitometric analysis of the results shows a 2.8 ± 0.4-fold increase in the expression of NHE1 in dDAVP-treated rats.
Effect of dDAVP on expression of AE1 in kidney outer medulla. We examined the expression of AE1, which colocalizes with SLC26A7 on the basolateral membrane of the OMCD (5, 24). In sharp contrast to SLC26A7, AE1 labeling is abundantly present in the OMCD of Brattleboro rats (Fig. 5A) and is not significantly affected by dDAVP (Fig. 5B). The number of cells expressing AE1 was 55 ± 4 cells per field (n = 1,105 cells per 20 fields per 4 animals) in vehicle-treated rats and 56 ± 3 cell per field (n = 1,121 cells per 20 fields per 4 animals) in dDAVP-treated rats (P > 0.05). Interestingly, dDAVP treatment decreased the intensity of AE1 labeling from 135.6 ± 4.6 (n = 1,105 cells per 20 fields per 4 animals) in vehicle-treated rats to 110.2 ± 3 (n = 1,121 cells per 20 fields per 4 animals, P < 0.005), consistent with a mild reduction in AE1 abundance by dDAVP.

Effect of dDAVP on expression of H^+-ATPase in kidney outer medulla. We examined the effect of dDAVP on the expression of H^+-ATPase, which is located on the apical membrane of α-intercalated cells. H^+-ATPase expression is abundant in the outer medulla of Brattleboro rats (Fig. 6A) and does not change significantly with dDAVP treatment (Fig. 6B). The number of cells labeled with H^+-ATPase was 45 ± 4 cells per field (n = 917 cells per 20 fields per 4 animals) in the outer medulla of vehicle-treated rats compared with 41 ± 4 cells per field (n = 826 cells per 20 fields per 4 animals) in dDAVP-injected rats (P > 0.05). The intensity of H^+-ATPase labeling was 128.1 ± 9.2 (n = 917 cells per 20 fields per 4 animals) in vehicle-treated rats and 134.5 ± 5.6 (n = 826 cells per 20 fields per 4 animals) in dDAVP-injected rats (P > 0.05).

dDAVP treatment has been shown to increase the plasma concentration of a number of hormones, including aldosterone. This latter effect is likely via activation of the renin-angiotensin-aldosterone axis (4, 29). In the last series of experiments, Sprague-Dawley rats were injected with DOCA (with or without saline load) for 4 days and compared with normal rats (with or without saline load). Treatment with saline and DOCA has been shown to cause significant hypokalemia, which, independent of DOCA and salt loading, can upregulate the expression of a number of acid-base transporters in cortical and medullary collecting ducts. The mean labeling intensity of 136.4 ± 9 (n = 560 cells per 10 fields per 2 animals) in vehicle-treated rats decreased to 91.4 ± 4.1 (n = 490 cells per 10 fields per 2 animals) in DOCA-treated rats (P < 0.0006). Similarly, the mean intensity of labeling was 141.5 ± 9.6 (n = 530 cells per 10 fields per 2 animals) in rats given 0.9% saline in their drinking water and injected with vehicle and 97.2 ± 4.4 (n = 590 cells per 10 fields per 2 animals) in salt-loaded rats injected with DOCA (P < 0.001). In summary, we found that DOCA with or without salt loading does not increase the expression of SLC26A7 and, indeed, DOCA may reduce the expression of SLC26A7.
DISCUSSION

The most salient feature of the present studies is the absence of expression of SLC26A7 in the OMCD of Brattleboro rats and its induction in response to dDAVP (Fig. 3). Treatment with dDAVP did not change the expression of H⁺-ATPase (Fig. 6).

The presence of two distinct Cl⁻/HCO₃⁻ exchangers, SLC26A7 and AE1, on the basolateral membrane of α-intercalated cells in OMCD (5, 24) raises the possibility of redundancy vs. differential regulation in pathophysiological states. Recent studies from our laboratory demonstrated that AE1 and SLC26A7 are differentially regulated in water deprivation (5), a condition that is associated with significant volume depletion, increased plasma antidiuretic hormone, and enhanced kidney AQP2 expression. It has been suggested that the upregulation of SLC26A7 in water deprivation could maintain the absorption of HCO₃⁻ in the medullary collecting duct in hypertonic environments, despite the downregulation of AE1 (5).

A basolateral Na⁺/H⁺ exchanger, most likely NHE1, has been identified in α-intercalated cells of OMCD (12, 40). Several studies have shown parallel adaptive regulation of Na⁺/H⁺ and Cl⁻/HCO₃⁻ exchange in epithelial and nonepithe-
induced by dDAVP (see RESULTS). We suggest that the reduction in its increase in response to dDAVP treatment are confirmed by the upregulation of SLC26A7 in Brattleboro rats was at the posttranscription level (Figs. 3 and 4). It is plausible that dDAVP treatment, either via increased interstitial osmolarity or some other signals, decreases the degradation or increases the half-life of SLC26A7 protein and, therefore, increases its abundance in the membrane. Published reports support the basis of this possibility: AQP1 half-life and expression increased in hypertonic media (40). In further support of this possibility, studies in murine inner medullary collecting duct (IMCD3) cells showed that although no detectable expression of the γ-subunit of Na⁺-K⁺-ATPase was observed in isotonic media, hypertonic media induced the plasma membrane expression of Na⁺-K⁺-ATPase (26). In direct relation to the role of osmolarity on SLC26A7 regulation, we observed that green fluorescent protein-tagged SLC26A7 is predominantly localized in transferrin-positive cytoplasmic endosomes when expressed in Madin-Darby canine kidney cells and examined in isotonic media but is detected exclusively in the plasma membrane after exposure to hypertonic media for 16 h (43). These latter observations clearly demonstrate that SLC26A7 shows adaptive posttranscriptional regulation in hypertonicity (43).

In conclusion, SLC26A7 protein expression is nearly abolished in the OMCD of Brattleboro rats, but it is induced in response to dDAVP and restored to levels observed in normal Sprague-Dawley rats. We propose that the induction of SLC26A7 by dDAVP is likely due to increased medullary interstitial osmolarity and may allow for the continuation of HCO₃⁻ absorption in the OMCD in light of the downregulation of AE1.

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REFERENCES

SLC26A7 REGULATION BY VASOPRESSIN IN OMCD


