Vesicular monoamine transporter 1 mediates dopamine secretion in rat proximal tubular cells

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Maurel A, Spreux-Varoquaux O, Amenta F, Tayebati SK, Tomassoni D, Seguelas M-H, Parini A, Pizzinat N. Vesicular monoamine transporter 1 mediates dopamine secretion in rat proximal tubular cells. Am J Physiol Renal Physiol 292: F1592–F1598, 2007. First published January 23, 2007; doi:10.1152/ajprenal.00514.2006.— Renal dopamine, synthesized by proximal tubules, plays an important role in the regulation of renal sodium excretion. Although the renal dopaminergic system has been extensively investigated in both physiological and pathological situations, the mechanisms whereby dopamine is stored and secreted by proximal tubule cells remain obscure. In the present study we investigated whether vesicular monoamine transporters (VMAT)-1 and -2, which participate in amine storing and secretion, are expressed in rat renal proximal tubules, and we defined their involvement in dopamine secretion. By combining RT-PCR, Western blot, and immunocytochemistry we showed that VMAT-1 is the predominant isoform expressed in isolated proximal tubule cells. These results were confirmed by immunohistochemistry analysis of rat renal cortex showing that VMAT-1 was found in proximal tubules but not in glomeruli. Functional studies showed that, as previously reported for VMAT-dependent amine transporters, dopamine release by cultured proximal tubule cells was partially inhibited by disruption of intracellular H⁺ gradient. In addition, dopamine secretion was prevented by the VMAT-1/VMAT-2 inhibitor reserpine but not by the VMAT-2 inhibitor tetrabenazine. Finally, we demonstrated that tubular VMAT-1 mRNA and protein expression were significantly upregulated during a high-sodium diet. In conclusion, our results show for the first time the expression of a VMAT in the renal proximal tubule and its involvement in dopamine secretion. These data represent the first step toward the comprehension of the role of this transporter in renal dopamine handling and its involvement in pathological situations.

DOPAMINE IS A BIOGENIC MONOAMINE playing a major role in regulation of renal sodium handling (5). This amine exerts natriuretic action by activating dopamine receptors located at the basolateral or apical side of the proximal tubule and of more distal segments of the nephron (3, 5). Activation of dopamine receptors reduces tubular sodium reabsorption by inhibition of two main sodium transport systems, the Na⁺-K⁺-ATPase and the Na⁺/H⁺ exchanger, located at the basolateral and apical membranes of the tubular cells, respectively (17). Synthesis of dopamine by the kidney occurs mainly in the proximal tubules. In this segment of the nephron, the dopamine precursor L-DOPA is internalized into the cells from the glomerular filtrate and plasma and then converted to dopamine by l-aromatic amino acid decarboxylase (l-AAAD) (10, 22, 24). Once synthesized, dopamine is transported out of the cell to activate tubular dopamine receptors. The amount of dopamine secreted depends, in part, on the activity of two catecholamine-degrading enzymes, monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) (18, 21, 27). Indeed, it has been shown that these enzymes are abundant in tubular cells and their inhibition leads to a significant increase in the availability of renal dopamine. Although the renal dopaminergic system has been extensively investigated in both physiological and pathological situations, the mechanisms whereby tubular dopamine is stored and secreted remain obscure.

Most of the information available on the mechanisms of monoamine neurotransmitter (dopamine, norepinephrine, and serotonin) storing and secretion concern the central nervous system. In neurons, newly synthesized monoamines are sequestered into vesicles. Intravesicular storing of monoamine appears to be involved in prevention of their chemical- and enzyme-mediated inactivation as well as in regulation of their secretion (11, 13). The accumulation of monoamines from the cytoplasm to storage organelles is mediated by vesicular monoamine transporters (VMATs). These transporters are subdivided into two major forms, VMAT-1 and VMAT-2, encoded by two distinct genes and differentiated on the basis of inhibition specificity and affinity for different amines. VMAT-1 and VMAT-2 show a high sequence similarity but appear differentially expressed in organs synthesizing amines (9, 16). The VMAT-1 isoform seems to be restricted to endocrine cells, whereas VMAT-2 is expressed in neurons and some neuroendocrine cells (7, 30). Transport of monoamines into secretory vesicles by VMATs requires a proton electrochemical gradient across the vesicle membrane that drives the exchange of one monoamine with two protons through the transporter protein. Experiments performed in genetically modified mice showed that VMAT is necessary not only for monoamine storing but also for their secretion. Indeed, concerning dopamine, it has been shown that VMAT-2-knockout mice have impaired neuronal dopamine storing and secretion (11, 28).

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In the present study we investigated whether VMATs are expressed in rat renal proximal tubule, and we defined their involvement in dopamine secretion.

EXPERIMENTAL PROCEDURES

Materials. HBSS medium, DMEM-Ham’s F-12 medium, nonessential amino acid mixture, penicillin, streptomycin, EGF, and FCS were from Invitrogen (France); Percoll was from Pharmacia (Sweden); polyvinylidene difluoride (PVDF) membranes were from NEN Life Science Products; rabbit polyclonal anti-VMAT-1 were purchased from Chemicon (Temecula, CA) and from Santa Cruz Biotechnology (Santa Cruz, CA). Blocking peptides corresponding to that used for generating antibody were also purchased from Chemicon. Goat polyclonal anti-VMAT-2 antibody and horseradish peroxidase (HRP)-conjugated anti-goat IgG were from Santa Cruz Biotechnology; ECL Kit detector reagents were from Amersham (Little Chalfont, UK); the biotin-streptavidin immunostaining kit was purchased from Vector Laboratories (Burlingame, CA). Bio-Rad DC protein assay reagents were from Bio-Rad Laboratories (Ivy-sur-Seine, France); other chemicals were purchased from Sigma (St. Louis, MO).

Isolation and primary culture of rat proximal tubule cells. Male Sprague-Dawley rats (250 g) were purchased from Harlan (France). A total of eight rats were divided into two groups and placed on either a high-salt (6% NaCl) or a normal-salt diet for 3 wk. They were housed two animals per cage and maintained under controlled conditions of light, temperature, and humidity. They were killed after 3 wk of diet, and proximal tubules were isolated as described below. All animal experiments were performed in accordance with the European Community guidelines for the use of experimental animals and national law. The animal ethics board of the Institut de Médécine Moléculaire de Rangueil approved all animal experimental procedures. The primary culture cells were isolated from Sprague-Dawley rats (40 g) as described by Vinay et al. (26). Briefly, rats were anesthetized with pentobarbital sodium, and the kidneys were removed aseptically, decapsulated, and minced coarsely in HBSS supplemented with 10 mM HEPES and 5 mM t-glucose, pH 7.4. Cortex was separated from the medulla and incubated in HBSS supplemented with 0.48 U/ml collagenase and 0.1% BSA in a flask under gentle stirring for 40 min at 37°C under a 5% CO2 atmosphere. To separate homogeneous populations of nephron segments, the mixture of tubules was suspended in 42% Percoll made isotonic with 10× concentrated Krebs-Henseleit buffer (1.18 M NaCl, 47 mM KC1, 100 mM HEPES, 200 mM cacodylic acid, 1.26 mM MgSO4, 11.4 mM KH2PO4, 50 mM glucose) and was centrifuged (17,000 rpm, 30 min, 4°C). The F4 layer, composed by proximal tubules, was suspended in culture medium (DMEM-Ham’s F-12 medium supplemented with 10 mM HEPES, 25 mM NaHCO3, 4 mM glutamine, 20 nM sodium selenite, 10 ml/l of a 100× nonessential amino acid mixture, 50 U/ml penicillin, 50 µg/ml streptomycin, 10 µg/ml insulin, 5 mM transferrin, 0.1 mM dexamethasone, 10 ng/ml EGF, and 5 µg/ml triiodothyronine). Isolated tubules were centrifuged and used for experiments or plated at 6 mg or 0.6 mg of protein in 150-mm Petri dishes or six-well plates, respectively, that had been coated with collagen type I from calf skin. FCS (5%) was added to the culture medium until the first change of medium (2 days after seeding). The experiments were carried out on day 5.

Western blot analysis. Isolated or confluent rat proximal tubule cells were lysed in buffer containing (mM) 50 Tris·HCl, pH 7.5, 150 NaCl, 1 EDTA, and 1 EGTA, with 1% Triton X-100, 10 µg/ml PMSF, 3 µg/ml aprotinin, and 3 µg/ml leupeptin. After sonication, proteins (60 µg of isolated or confluent rat proximal tubule cells and 40 µg of adrenal gland or brain) were loaded on a 10% polyacrylamide gel and transferred to PVDF membrane. The membrane was blocked with 1% BSA in Tris-buffered saline (TBS)-Tween 20 (0.1%) overnight at 4°C. Polyclonal anti-VMAT-1 (1:500), anti-VMAT-2 (1:500), or anti-actin (1:1,000) was used as primary antibody. After incubation with appropriate HRP-linked secondary antibody (1:10,000, 30 min, room temperature), proteins were detected with the ECL reaction. The blot was stripped completely of antibodies before reprobing with a polyclonal anti-actin. Protein was measured with a Bio-Rad protein assay kit with gamma globulin as a standard.

RNA isolation, RT-PCR, and real-time PCR analysis. Total RNA was extracted from proximal tubule cell culture and from isolated proximal tubules with the Qiagen RNA extraction kit according to the manufacturer’s instructions.

First-strand cDNA was synthesized from up to 1 µg of total RNA by reverse transcription for 60 min at 42°C in a final volume of 20 µl of reverse transcription buffer with 100 U of Superscript II (Invitrogen), 0.25 µg of oligo(dT)12–18, 0.5 mM dNTPs, 5 mM DTT, and 32 U of RNase inhibitor. Five microliters of first-strand cDNA was then used to amplify VMAT-1 and VMAT-2 fragments by PCR. A reaction mix containing PCR buffer (1.5 mM MgCl2, 0.2 mM dNTPs, 60 nM primers, and 2 U of Taq polymerase) and the reverse transcription reaction product was denatured at 93°C for 2 min 30 s, and consequently VMAT-1 and -2 were amplified by 35 cycles with a DNA thermal cycler (TRIO-Thermoblock Biometra, Göttingen, Germany). A cycle was composed of a denaturation step at 95°C for 1 min, an annealing step at 56°C, and an extension step at 72°C for 1 min. The final extension step was prolonged to 10 min. The absence of contaminants was checked by RT-PCR assays of negative control samples in which the Superscript was omitted.

The primers used for VMAT-1 were defined by bases 134 5′-CCAGCCGACACTTCTTCTTCTATAA-3′ (forward) and 1938 5′-GACATTTACAGGTAGTAAGAGGATGTA-3′ (reverse) and the primers for VMAT-2 by bases 5′-CGGCGGCACTTCTTTATCT-3′ (forward) and 5′-CGGCGGCACTTCTTTATCT-3′ (reverse). The expected size of the amplification product was 1,831 and 405 bp for VMAT-1 and VMAT-2, respectively.

Real-time PCR analysis was performed in 96-well plates with an ABI Prism 7000 HT Sequence Detection System (Applied Biosystems) and SYBR Green PCR Master Mix (Applied Biosystems). Amplification reactions (25 µl) were carried out in triplicate with 5 µl of 1:5 diluted template cDNA according to the manufacturer’s protocol (Applied Biosystems). Each assay was normalized by amplifying the housekeeping cDNA 18S from the same cDNA sample. The parameters included a single cycle of 94°C for 10 min followed by 40 cycles of 95°C for 15 s, annealing, and 60°C for 1 min. The primers were VMAT-1 forward: 5′-CTAAGGGAGGAAGGCTGCAA-3′; VMAT-1 reverse: 5′-CGGTGTTTCTCCTAGTGGAAAC-3′; VMAT-2 forward: 5′-AGCTTCCGACTTAAATTGAC-3′; and VMAT-2 reverse: 5′-CACTAAGAACGCCGTATGCA-3′.

Assay of dopamine. Cell monolayers, seeded into six-well plates, were washed and preincubated or not with reserpine, carbonyl cyanide p-trifluoromethoxyphenylhydrazine (FCCP), or tetrabenzine for 20 min at 37°C before i-DOPA was added. The MAO inhibitor pargyline (10−3 M) and the COMT inhibitor tolcapone (10−6 M) were added in cell medium 20 min before the experiment to preserve dopamine from enzymatic degradation. Cells were then incubated with increasing concentrations of i-DOPA in HBSS for 1 h at 37°C. The inhibitors were present during the entire period of time. At the end of the incubation period, the medium was removed to measure extracellular dopamine. One milliliter of cell supernatant was added to 25 µl of 1 N HClO4, snap frozen, and stored at −80°C. The assays for dopamine were performed by a specific and sensitive high-pressure liquid chromatography method with coulometric detection, applied to cell supernatants, similar to that of Alvarez et al. (1).

Immunocytochemistry and immunohistochemistry. We performed experiments with two different sources of VMAT-1 antibodies, one from Santa Cruz Biotechnology (sc7720) and another from Chemicon (AB1597P). Proximal tubule cells, cultured on coverslips, were fixed in 4% paraformaldehyde in TBS (mM: 137 NaCl, 2.68 KCl, 25 Tris, pH 7.4) for 10 min at 4°C. After permeabilization with 0.3% Triton X-100 in TBS, cells were incubated 30 min with 1% FCS in TBS.
and then incubated with rabbit polyclonal anti-VMAT-1 antibody (Chemicon) or rabbit anti-VMAT-2 antibody (1:100 dilution) for 12 h at 4°C. After being washed, the slices were incubated with affinity-purified FITC-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories; 1:500) in TBS for 1 h and mounted with mounting medium for fluorescence (Dako). The second protocol was realized with VMAT-1 antibodies from Santa Cruz Biotechnology. After incubation with the rabbit polyclonal anti-VMAT-1 antibody, cells were rinsed twice in TBS buffer and exposed for 30 min at 25°C to a biotinylated anti-rabbit secondary antibody at a dilution of 1:200. The product of the immune reaction was then revealed with a biotin-streptavidin immunostaining kit with 0.05% diaminobenzidine (DAB) in 0.1% H2O2 as a chromogen. The antibodies were detected at the appropriate wavelength by confocal microscopy (Zeiss), nonspecific background being subtracted where primary antibody was omitted.

Male Sprague-Dawley rats were anesthetized with pentobarbital sodium and perfused through the abdominal aorta with a 0.9% NaCl solution containing heparin (20 IU/100 ml). This solution was replaced by a fixative solution of 4% freshly prepared paraformaldehyde and 0.4% picric acid in phosphate buffer (pH 6.9). At the end of perfusion, the kidneys were removed. Renal cortices were separated from medulla, embedded in cryoprotectant medium [optimum cutting temperature (OCT)], and immediately frozen at −50°C. OCT blocks were cut serially with a motorized cryostat microtome and mounted on polylysine-treated microtome slides. Groups of five consecutive slides containing one 10-μm-thick section were used. The first section was stained with Masson’s trichrome technique to verify microanatomic details. The second section was processed for the histochemical detection of alkaline phosphatase as a marker of proximal tubules according to Gomori’s metal salt method (see Ref. 25). The third section was exposed to the primary antibody anti-VMAT-1 from Chemicon in phosphate buffer containing 0.2% (wt/vol) BSA, 0.03% Triton X-100, and 0.1% (wt/vol) sodium azide, diluted 1:500. The fourth section was incubated as above with the primary anti-VMAT-1 antibody preadsorbed with the peptide antigen purchased from Chemicon. Incubation with antibody was accomplished in a humid chamber at 4°C for 12–18 h. Optimal antiserum dilutions and incubation times were assessed in a series of preliminary experiments. After incubation, slides were rinsed twice in phosphate buffer and exposed for 30 min at 25°C to a biotinylated anti-rabbit secondary antibody at a dilution of 1:200. The product of the immune reaction was then revealed with a biotin-streptavidin immunostaining kit with 0.05% DAB in 0.1% H2O2 as a chromogen. Sections were then washed, dehydrated in ethanol, and mounted in a synthetic mounting medium.

Statistical analysis. Dose-response curves of dopamine release were evaluated with nonlinear square curve-fitting procedures (Prism GraphPad, San Diego, CA). Results are expressed as means ± SE. The statistical significance of differences between two experimental groups was evaluated by Student’s t-test. A P value of <0.05 was considered significant.

Fig. 1. Expression of vesicular monoamine transporters (VMATs) in rat proximal tubules. A: amplification by RT-PCR in adherent proximal tubular cells and isolated proximal tubules of a DNA fragment corresponding to VMAT-1 (left) or VMAT-2 (right). MW, 1-kb DNA ladder. B: Western blot analysis of VMAT-1 and VMAT-2 protein. Protein extracts of adherent proximal tubular cells, isolated proximal tubules, adrenal gland, or brain were separated by SDS-PAGE. Arrow shows predominant bands of ~55 kDa (VMAT-1) and 68 kDa (VMAT-2).
RESULTS

Expression of VMATs in rat proximal tubules. To verify the existence of VMATs in proximal tubule, we first determined whether VMAT mRNAs were expressed in primary cultures of proximal tubule cells by RT-PCR analysis. Two primer pairs designed to amplify coding sequences of VMAT-1 and VMAT-2 isoforms were used. As shown in Fig. 1A, both VMAT-1 and VMAT-2 DNA fragments were amplified in cultured proximal tubule cells. Similar results were obtained when total RNA from isolated proximal tubules was used as a template for RT-PCR amplification (Fig. 1A). The nucleotide sequences of the amplified fragments completely matched VMAT-1 or VMAT-2 sequence.

Next, we addressed the expression of VMAT protein by two approaches: immunoblot and immunocytohistochemistry analysis. For immunoblots, adrenal and brain were used as positive control for VMAT-1 and VMAT-2, respectively. Immunoblots with anti-VMAT-1 antibodies revealed the immunodetection of a band of ~55 kDa in isolated proximal tubules, primary culture of proximal tubules, and brain (Fig. 1B). In contrast, immunoblots with anti-VMAT-2 antibodies did not reveal immunoreactive bands in isolated proximal tubules or primary culture of proximal tubules (Fig. 1B).

Immunocytochemistry analysis with VMAT-1 antibodies performed with two different sources of antibodies on primary culture of proximal tubules revealed a strong signal found in the totality of cells present in microscope slides. Confocal laser microscopy showed that immunoreactivity was observed within cell cytoplasm as particle-like structures, suggesting that VMAT-1 protein was associated with intracellular organelles (Fig. 2). As previously observed for immunoblots, VMAT-2 immunoreactivity was not detectable in adherent primary culture of proximal tubule cells (data not shown). These data suggest that VMAT-1 is the predominant form of vesicular transporter expressed both in primary culture and isolated proximal tubule cells.

Expression of VMAT-1 in proximal tubules was confirmed by immunohistochemistry analysis. Indeed, experiments performed on tissue preparations from rat renal cortex showed that a high expression of VMAT-1 was found in proximal tubules but not in glomeruli or other cortical convoluted tubules (Fig. 3). The pattern of immunostaining was abolished in slides incubated with antibody preabsorbed with the blocking peptide (Fig. 3D).

Synthesis and release of dopamine by proximal tubule cells. To investigate the functional relevance of VMAT-1, we first measured synthesis of dopamine release in supernatant of adherent cultured proximal tubule cells incubated with increasing concentrations of L-DOPA for 1 h. The concentration of dopamine released in extracellular medium was saturable and dependent on L-DOPA concentration added to the medium (Fig. 4A). To determine whether VMAT-1 protein participates in dopamine secretion in the extracellular compartment we incubated cells with different pharmacological agents. Previous studies have shown that disruption of H+/H2O gradient abrogates amine storage (8) and neurotransmission. In light of these results, we investigated the effect of the protonophore FCCP on dopamine release. FCCP partially prevented dopamine release (26–32%) in extracellular medium (Fig. 4B), indicating that proton gradient is necessary to efficient secretion of newly synthesized dopamine. To identify the VMAT isoform involved in dopamine secretion, cells were incubated with the VMAT isoform involved in dopamine secretion, cells were incubated with the VMAT

Fig. 2. Immunolocalization of VMAT-1 in proximal tubules of rat renal cortex. Confocal images, obtained from primary culture of proximal tubular cells, were taken with ×100 lens. Experiments were performed with 2 different sources of antibodies. A: cells were labeled with anti-VMAT-1 antibodies from Santa Cruz Biotechnology and revealed with a biotinylated anti-rabbit secondary antibody and a biotin-streptavidin immunostaining kit as described in EXPERIMENTAL PROCEDURES. The negative control without the primary antibody (left) showed no staining. B: cells were double-labeled with antibodies from Calbiochem against VMAT-1 (green) and the nuclear stain propidium iodide (red). Background fluorescence was eliminated by setting the thresholds with parallel preparations stained under identical conditions but without primary antibodies (left). Images are representative of 3 independent experiments.
inhibitors reserpine and tetrabenzine. As shown in Fig. 4B, cell incubation with the VMAT-1/VMAT-2 inhibitor reserpine inhibited by 45% L-DOPA-dependent dopamine secretion by proximal tubule cells. In contrast, dopamine release was not affected by the VMAT-2 inhibitor tetrabenzine. These data suggest that VMAT-1 participates in regulation of newly synthesized dopamine from proximal tubule cells.

**Effect of high-salt diet in tubular VMAT-1 expression.** Previous studies showed that the expression of the dopamine-synthesizing enzyme L-AAAD in proximal tubules was regulated by sodium intake (15, 23). To determine whether VMAT-1 expression could also be modulated by dietary sodium, we investigated the effect of chronic sodium loading on VMAT-1 mRNA and protein expression in proximal tubuli. VMAT-1 expression was quantified in rats fed with a normal (0.63%) or high (6%)-NaCl diet for 3 wk. As shown in Fig. 5, VMAT-1 mRNA and protein were increased by 1.7- and 3-fold, respectively, after the high-sodium diet.

**DISCUSSION**

During the last years, several studies have described different components of the renal proximal tubule dopaminergic system including the transporter of the dopamine precursor L-DOPA, the dopamine-synthesizing enzyme L-AAAD, and the dopamine-degrading enzymes MAO and COMT. These proteins have been implicated to different extents in regulation of dopamine activity in kidney. In the present work, we identified for the first time VMAT-1 as a component of the tubular dopaminergic system and we showed its role in dopamine release from proximal tubules.

The expression of VMAT-1 in proximal tubules was shown by complementary results. Indeed, VMAT-1 mRNA and protein were detected both in isolated and primary cultures of proximal tubules from rat kidney. Expression of VMAT-1 protein in proximal tubules was confirmed by immunohistochemistry studies. Interestingly, VMAT-1 could not be localized in glomeruli. This finding is in agreement with previous studies showing that synthesis and secretion of renal dopamine mainly occur in the proximal tubule (14). As previously reported for VMATs in neuronal and chromaffin cells, confocal microscopy showed that VMAT-1 was compartmentalized in the cytosol. The functional relevance of VMAT-1 in dopamine handling was shown by studies of dopamine synthesis and release by proximal tubule cells. In these studies, we demonstrated that cultured proximal tubule cells were able to produce and secrete dopamine from exogenous L-DOPA. As classically described for the general mechanism of VMAT-dependent amine storing in secretory vesicle, disruption of intracellular H⁺ gradient significantly reduced dopamine release by proximal tubule cells. Dopamine secretion was partially inhibited by the VMAT-1/VMAT-2 inhibitor reserpine and was non affected by the VMAT-2 inhibitor tetrabenzine. Together, biochemical, morphological, and functional studies show that
VMAT-1 is the predominant isoform of vesicular transporter involved in tubular dopamine secretion. These results differ from those obtained in the central nervous system, where dopamine storing and secretion are regulated by VMAT-2. In the kidney, the role of this transporter in dopamine handling cannot be completely ruled out, because mRNA encoding for VMAT-2 was found by RT-PCR. However, the expression of VMAT-2 protein could not be confirmed by Western blot, immunocytochemistry, and functional studies. In addition, VMAT-1 and VMAT-2 are usually considered to be mutually exclusive except in chromaffin cells and human lymphocytes, where both transporters are expressed in the same cell type (2, 20).

Fig. 4. Inhibition of dopamine release from proximal tubular cells. A: dose-dependent effect of L-DOPA on dopamine secreted in culture medium of proximal tubule cell primary culture. Cells were incubated with various concentrations of L-DOPA for 60 min. Data are means ± SE of 4 separate experiments. B: effect of pharmacological inhibitors on dopamine accumulation in extracellular medium. Cells were preincubated with carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP, 2.5 μM) or VMAT inhibitors reserpine (4 μM) and tetrabenazine (2 μM) for 20 min before addition of L-DOPA. Dopamine content was measured in culture medium after 60-min incubation. Data are expressed as % of control and are means ± SE of 2–6 separate experiments. *P < 0.05, **P < 0.01, ***P < 0.001, compared with NS values.

Fig. 5. Effect of high-salt diet on VMAT-1 expression in rat proximal tubules. A: VMAT-1 mRNA expression was determined by real-time RT-PCR and normalized by 18S rRNA in isolated proximal tubules of Sprague-Dawley rats fed a normal-salt (0.63% NaCl, NS) or a high-salt (6% NaCl, HS) diet (n = 4 in each group). B: Western blot analysis were performed on isolated proximal tubule extracts (60 μg) of Sprague-Dawley rats submitted to a NS or a HS diet (n = 4 in each group). Histogram represents the ratio of optical densities (ODs) corresponding to VMAT-1 and actin bands. **P < 0.005 compared with NS values. *P < 0.05 compared with NS values.
The renal dopaminergic system seems to be highly dynamic and regulated by different factors. Indeed, a positive correlation has been described between sodium intake and renal dopamine production/excretion in both humans and laboratory animals (6, 12, 19). Urinary dopamine, which is believed to reflect renal dopamine synthesis, is increased during high salt intake and during extracellular fluid volume expansion induced by saline infusion. The enhanced dopamine synthesis and secretion contribute to the increase in natriuresis through the dopamine-receptor dependent inhibition of tubular sodium reabsorption. Changes in renal dopamine during high salt intake depend, in part, on the increase in availability of L-DOPA and the increase in L-AAAD activity. In addition to these events, it has been shown that sodium loading induces a different polarization of dopamine secretion at the apical and basolateral sides of tubular cells. Indeed, in basal conditions, excretion of dopamine in vivo was found to be about threefold higher in urine than in renal interstitial fluid of intact kidney (4). During chronic sodium loading or acute infusion of gludopa, only urinary excretion of dopamine was increased, whereas dopamine concentration of renal institial fluid remained unchanged (29). These results suggested that sodium loading could regulate the mechanisms of dopamine secretion in the proximal tubuli.

Following this hypothesis, we investigated the effect of high-sodium diet on VMAT-1 expression in proximal tubules. Our results showing that chronic sodium loading increased significantly both VMAT-1 mRNA and protein support the possibility that sodium intake regulates not only dopamine synthesis but also the expression of proteins involved in its secretion. It is conceivable that changes in VMAT-1 expression may contribute to the modification of polarity of dopamine secretion observed during sodium loading. Further studies will be necessary to confirm this possibility.

In conclusion, our results show for the first time the expression of a VMAT in the renal proximal tubule and its involvement in regulation of dopamine secretion. These data represent the first step toward the comprehension of the role of this transporter in renal dopamine handling and its role in pathological situations.

REFERENCES


