Dopamine is a natriuretic hormone that acts by inhibiting tubular Na⁺,K⁺-ATPase activity by activation of the dopamine-1 receptor (the thick ascending limb [TAL] of Henle) or by a synergistic effect of dopamine-1 and dopamine-2 receptors (the proximal tubule). The dopamine-1 receptor is coupled to adenylate cyclase. In this article we show that prehypertensive Dahl salt-sensitive (DS) rats have a blunted natriuretic response to dopamine determined during euvolemic conditions compared with Dahl salt-resistant (DR) rats. Basal Na⁺,K⁺-ATPase activity was similar in DS and DR rats. In proximal tubule, dopamine (10⁻³ M) inhibited Na⁺,K⁺-ATPase activity in DR but not in DS rats. The dopamine-2 agonist LY171555 (10⁻⁵ M) together with dibutyryl cyclic AMP (10⁻⁴ M) inhibited proximal tubule Na⁺,K⁺-ATPase activity in both DS and DR rats. LY171555 alone had no effect. In TAL, the dopamine-1 agonist fenoldopam (10⁻³ M) inhibited Na⁺,K⁺-ATPase activity in DR but not in DS rats. Dibutyryl cyclic AMP (10⁻⁴ M) inhibited TAL Na⁺,K⁺-ATPase activity in both DS and DR rats. In cell suspensions from the cortex and the medulla, activation of the dopamine-1 receptor significantly increased cyclic AMP content in DR but not in DS rats. The results indicate that DS rats lack the capacity to inhibit tubular Na⁺,K⁺-ATPase activity because of a defective dopamine-1 receptor adenylate cyclase coupling. This defect may contribute to the impaired natriuretic capacity in DS rats.

KEY WORDS • Na⁺,K⁺-transporting ATPase • rats, inbred strains • dopamine • receptors, dopamine D1 • adenosine cyclic monophosphate • natriuresis • hypertension, sodium-dependent

Methods

Animals

Studies were performed on male prehypertensive DS (SS/Jr) and DR (SR/Jr) rats aged 7–8 weeks and weighing 160–240 g. These rats were purchased from Mollegaards Breeding Center, Ejby, Denmark. The rats receiving a normal salt diet were fed a standard rat chow (R3, Ewos, Sodertalje, Sweden) that contains 0.7% NaCl and received tap water ad libitum. Rats were anesthetized with Inactin-Byk (Byk-Gulden, Konstanz, FRG) (80 mg/kg body wt i.p.). Mean arterial pressure was recorded via one carotid artery. Inactin-Byk anesthesia does not influence mean arterial pressure, as shown previously. Mean arterial pressure values were similar in DS and DR rats on a normal salt diet (116±3 versus 110±2 mm Hg).

Preparation of Tubules

Kidney perfusion and tubule microdissection were performed as described. After a midline incision, the left kidney was exposed and perfused with the following modified Hanks' solution (mM): NaCl 137, KCl 5, MgSO₄,
ATP hydrolysis. Na+,K+-ATPase activity was calculated as phosphate released from [γ-32P]ATP in six to eight segments was determined to correct for nonspecific uptake on activated charcoal. The radioactivity was measured after absorption of unhydrolyzed nucleotide through a Millipore filter. Butyrate (10^{-3} M) was also added to the microdissection solution to optimize the mitochondrial respiration.

Microdissection was performed on ice under microscopic observation. The PT were manually dissected from the outer cortex and the TAL from the outer medulla. They were individually transferred to the concavity of the bacteriological slide and photographed for length determination in an inverted microscope at ×100 magnification. The tubules were stored on ice for 30 minutes.

Incubation of Tubules With Drugs

Tubules were incubated for 30 minutes at room temperature either in 1 μL microdissection solution alone (control tubules) or in 1 μL microdissection solution containing one or more of the following drugs (experimental tubules): dopamine (Kali-Chemie, Hanover, FRG), the DA-1 agonist fenoldopam (Smith-Kline & French Laboratories, Philadelphia, Pa.), dibutyryl cAMP (Sigma), and the DA-2 agonist LY171555 (Eli Lilly and Co., Indianapolis, Ind.). Drug doses were selected on the basis of previous experience.\(^\text{15}\) In Sprague-Dawley rats, dopamine inhibition of tubular Na+,K+-ATPase activity generally becomes apparent at 5×10^{-7} to 10^{-6} M. A dose of 10^{-5} M gives a maximal response. Values for experimental tubules were expressed as percent change from control tubules.

Determination of Na^+,K^+-ATPase Activity

Na^+,K^+-ATPase activity was measured as described previously.\(^\text{15}\) The tubular segments were made permeable by hypotonic shock, freezing, and thawing. Individual segments were incubated for 15 minutes at 37°C in a medium containing (mM) NaCl 50, KCl 5, MgCl\(_2\) 10, EGTA 1, Tris-HCl 100, and Na\(_2\)ATP 10 (grade II, Sigma) as well as 2–5 mM [γ-32P]ATP (New England Nuclear, Boston) in tracer amounts (5 nCi/μL). For determination of ouabain-insensitive ATPase (Mg-ATPase) activity, NaCl and KCl were omitted, Tris-HCl was 7.4. The phosphate liberated by hydrolysis of [γ-32P]ATP was separated by filtration through a Millipore filter after absorption of unhydrolyzed nucleotide on activated charcoal. The radioactivity was measured in a liquid scintillation spectrophotometer.

In each study, we determined total ATPase activity and Mg-ATPase activity in six to eight segments. The phosphate released from [γ-32P]ATP in six to eight samples of incubation solution containing no tubule segments was determined to correct for nonspecific ATP hydrolysis. Na^+,K^+-ATPase activity was calculated as the difference between the mean values for total ATPase activity and for Mg-ATPase activity.

Preparation of Cortical and Medullary Tubule Cell Suspensions

The tubule cell suspensions were prepared as described previously. Briefly, the cortex or outer medulla, which mainly contains TAL segments,\(^\text{14}\) was minced on ice and incubated in Dulbecco's modified Eagle's medium containing 0.065% collagenase at 37°C for 60 minutes. During incubation, the solution was continuously exposed to 95% oxygen and 5% carbon dioxide. The cortical cell suspension was poured through graded filters (pore size: 180, 75, 53, and 38 μm) to remove the glomeruli. The isolated cells were washed three times by centrifugation at 100g for 4 minutes. The preparations were magnified and inspected. They mainly consisted of free cells but also a few tubular segments. To assess the viability of tubule cells, we incubated the cells with 0.15% trypan blue for 5 minutes. Trypan blue staining occurred in <5% of the cells.

To check that the ATP production was adequate and not different in DS and DR rats, total and ouabain-sensitive oxygen consumption were measured under various conditions for Na^+. We found no difference between the strains.

In one protocol, cortical tubule cell suspensions were prepared from DS rat kidney and denervated for 2 days. The left kidney was denervated as described previously.\(^\text{19}\) The renal artery was dissected free from surrounding tissue and wrapped three times for 5 minutes with cotton soaked in a solution of 10% phenol in absolute alcohol.

Cyclic AMP Assay

Aliquots (100 μL) of tubule cell suspensions were transferred to 400 μL Dulbecco's modified Eagle's medium containing 10^{-3} M butyrate and drugs to be tested. Cells were incubated for 2 minutes at 37°C in the presence of 1 mM 3-isobutyl-1-methylxanthine. In this condition, the time course of cAMP accumulation was linear in both cortical and medullary tubule cell preparations. The reaction was terminated by the addition of ice-cold trichloroacetic acid to a final concentration of 6%. The cells were sonicated without changing the medium. Samples were then extracted with water-saturated ether and were subsequently assayed for cAMP content using a radioimmunoassay kit (New England Nuclear).

Glomerular Filtration Rate and Urinary Sodium Excretion

Rats were anesthetized with Inactin-Byk. Tracheostomy was performed to ensure a free airway during the experiment. One carotid artery (blood sampling) and two jugular veins (infusion) were catheterized. Both ureters were catheterized with thin polyethylene tubing for urine collection. Normal saline containing 5% Inustest (Laevosan, Linz, Austria) was given at a rate corresponding to 1% of the body weight per hour. It is our experience, as judged by numerous determinations of hematocrit and blood pressure, that this infusion rate will maintain the rats in a euolemic state. After a priming period of 1 hour, urine collection was started. After one control period, dopamine was added in a dose of 0.1 μg/100 g body wt per minute. Plasma and urine were analyzed for inulin as described previously\(^\text{20}\) and...
for sodium with a flame photometer (Eppendorf 6524, Hamburg, FRG).

**Statistical Analysis**

Values are given as mean±SEM. Statistical analysis was performed with the paired and unpaired Student's t test and analysis of variance. A value of \( p < 0.05 \) was considered significant.

**Results**

Glomerular filtration rate measured under euvoletic conditions was not significantly different in DS and DR rats on a normal salt diet (2.05±0.21 and 2.37±0.10 mL/min, respectively) and was not affected by dopamine in either strain. Absolute sodium excretion (micromole sodium per minute) was similar in DS and DR rats under basal conditions (0.67±0.16 and 0.60±0.13, respectively). During dopamine infusion, sodium excretion was increased significantly in DR (1.22±0.18) but not in DS (0.76±0.06) rats. Fractional sodium excretion, which was similar in DS and DR rats (0.26±0.02% versus 0.16±0.08%) under basal conditions, was also significantly increased in response to dopamine in DR rats (76.8±17.2% over basal, \( p < 0.05 \)) but not in DS rats (11.7±9.7% over basal) (Figure 1).

\( \text{Na}^+,\text{K}^+\text{-ATPase activity in TAL was similar in DS and DR rats on a normal salt diet (4,148±168 versus 4,473±295 pmol P_j per millimeter of tubule per hour). The DA-1 agonist fenoldopam significantly inhibited Na}^+,\text{K}^+\text{-ATPase activity in DR rats. The effect was dose dependent. The highest concentration of fenoldopam used, 10}^{-5} \text{M, caused 19.6±4.2% inhibition (} p < 0.05 \text{). Fenoldopam had no effect in DS rats (Figure 2). Mg-ATPase activity in TAL was similar in DS and DR rats and was not influenced by fenoldopam (data not shown).} \]

\( \text{Na}^+,\text{K}^+\text{-ATPase activity in PT was also similar in DS and DR rats on a normal salt diet (4,148±168 versus 4,473±295 pmol P_j per millimeter of tubule per hour). Dopamine (10}^{-5} \text{M} \text{) significantly inhibited Na}^+,\text{K}^+\text{-ATPase activity in DR rats (16.2±5.0% over basal, } p < 0.05 \text{) but not in DS rats (1.3±7.0% over basal) (Figure 3). Mg-ATPase activity in PT was similar in DS and DR rats and was not influenced by dopamine (data not shown).} \]

\( \text{Dibutyryl cAMP (10}^{-5} \text{M) significantly inhibited Na}^+,\text{K}^+\text{-ATPase activity in TAL in both DS (22.4±3.1% over basal, } p < 0.01 \text{) and DR (17.1±4.1% over basal, } p < 0.05 \text{) rats on a normal salt diet (Figure 4A). Dibutyryl cAMP did not affect Mg-ATPase activity in TAL from DS and DR rats (data not shown). Because DA-1 and DA-2 receptors synergistically inhibit Na}^+,\text{K}^+\text{-ATPase activity in TAL, the effect of dibutyryl cAMP (10}^{-6} \text{M) on Na}^+,\text{K}^+\text{-ATPase activity in PT was examined in the presence of the DA-2 agonist LY171555 (10}^{-5} \text{M) (Figure 4B). Under these conditions, Na}^+,\text{K}^+\text{-ATPase activity was significantly inhibited in both DS (12.2±2.8% over basal, } p < 0.05 \text{) and DR (15.7±0.8% over basal, } p < 0.01 \text{) rats. Dibutyryl cAMP and LY171555 did not affect the Mg-ATPase activity in either DS or DR rats (data not shown). Na}^+,\text{K}^+\text{-ATPase activity in PT from DR rats was not inhibited by the DA-1 agonist fenoldopam (10}^{-5} \text{M) alone, and Na}^+,\text{K}^+\text{-ATPase activity in PT from DS rats was not affected by either dibutyryl cAMP (10}^{-5} \text{M) or the DA-2 agonist LY171555 (10}^{-5} \text{M) alone (data not shown).} \)
The effect of dopamine receptor activation on cAMP content was studied in renal cell suspensions from DS and DR rats on a normal salt diet. Basal cAMP content was similar in DS and DR rats in the outer medulla (187±26 versus 207±65 pmol/mg protein per 2 minutes) as well as in the cortex (62.7±6.2 versus 71.4±16.7 pmol/mg protein per 2 minutes). In DR rats, fenoldopam significantly increased cAMP content (percent over basal) in suspended outer medullary cells (37.6±15.3% at 10⁻⁷ M and 56.7±7.6% at 10⁻⁵ M) (Figure 5). The increase in cAMP content caused by fenoldopam (10⁻⁵ M) was blocked by the DA-1 antagonist Sch 23390 (10⁻⁴ M) (4.6±4.6% over basal). In contrast, cAMP content did not increase in response to fenoldopam in DS rats. We found similar effects in cortical tubular cells (Figure 6). Fenoldopam (10⁻⁵ M) significantly increased cAMP content in DR rats (39.3±5.4% over basal) but not in DS rats. The fenoldopam (10⁻³ M)-induced increase in cAMP content in DR rats was blocked by the DA-1 antagonist Sch 23390 (10⁻⁴ M) (7.0±6.2% over basal). Dopamine also increased cAMP content in DR but not in DS rats. Forskolin, which directly stimulates adenylate cyclase, significantly increased cAMP content (percent over basal) to the same levels in DS (49.6±11.7% at 10⁻⁷ M and 586.6±55.5% at 10⁻⁵ M) and DR (52.2±7.9% at 10⁻⁷ M and 565.9±39.4% at 10⁻³ M) rats, confirming the observations by Felder et al.13

Because it has been suggested that increased activity of the sympathetic nervous system might contribute to salt-induced hypertension in DS rats,21 the effect of the sympathetic nervous system on adenylate cyclase activity was examined in cortical tubule cells from DS rats after adrenergic blockade by renal denervation. Basal cAMP content did not change or increase in response to 10⁻³ M fenoldopam in denervated DS rats.

Discussion

Dopamine is formed in proximal tubular cells. It acts as an autocrine or paracrine natriuretic factor by inhibiting the activity of Na⁺,K⁺-ATPase1⁴ and other ion transporters, such as the Na⁺-H⁺ exchanger.22 We now show that DS rats are insensitive to the effects of dopamine on tubular Na⁺,K⁺-ATPase activity and that DS rats are, under euvolemic conditions, insensitive to the natriuretic effect of dopamine. This dopamine insensitivity cannot be secondary to high blood pressure, because it was observed in normotensive DS rats on a normal salt diet.

Activation of adenylate cyclase, coupled to DA-1 receptor, and cAMP accumulation play a key role in dopamine inhibition of Na⁺,K⁺-ATPase activity.3⁵ In the PT,
Na⁺,K⁺-ATPase activity is inhibited by the synergistic actions of DA-1 and DA-2 receptors or by a DA-2 agonist and dibutyryl cAMP. In the TAL, Na⁺,K⁺-ATPase activity is inhibited by activation of DA-1 receptor alone or by forskolin and dibutyryl cAMP. Camp-dependent protein kinase may directly inhibit Na⁺,K⁺-ATPase activity by phosphorylating the α-subunit of the enzyme. Camp-dependent protein kinase may also indirectly inhibit Na⁺,K⁺-ATPase activity by phosphorylating a dopamine and cAMP-dependent phosphatase inhibitor, DARPP-32. Our data indicate that the lack of dopamine regulation of tubular Na⁺,K⁺-ATPase activity in DS rats is due to a defective coupling of DA-1 receptor to the adenylyl cyclase unit in the PT as well as in the TAL. We found that neither dopamine nor the DA-1 agonist fenoldopam stimulated adenylyl cyclase in renal tissue from the cortex or from the medulla. Similar observations have been reported by Felder and colleagues, who have extensively studied the coupling of DA-1 receptor to adenylyl cyclase in the PT of spontaneously hypertensive rats. Furthermore, we could show that inhibition of Na⁺,K⁺-ATPase activity occurred in both the PT and TAL of DS rats when a Camp analogue was present in the incubation medium. The mechanism for a defective coupling between the DA-1 receptor and adenylyl cyclase remains to be clarified. We could exclude that it is the result of increased renal nerve activity.

The data presented in this study also indicate that the lack of a dopamine effect on tubular Na⁺,K⁺-ATPase activity contributes to the blunted natriuretic capacity in DS rats. Dopamine significantly increased the fractional sodium excretion in DR rats but had no effect in DS rats. Several clinical observations in humans and experimental animals indicate that the natriuretic effect of endogenous dopamine is important for the maintenance of normal salt and water balance during a high salt diet. PT Na⁺,K⁺-ATPase activity is downregulated by a high salt diet in Sprague-Dawley and DR rats but not in DS rats. Dopamine produced in the kidney appears to contribute to this downregulation.

The studies presented here explain some of the cellular mechanisms underlying the lack of response to a high salt diet in DS rats. Important questions to be addressed in future studies are to identify the underlying molecular defects and the therapeutic strategies that will correct these defects.

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