Dopamine-dependent inhibition of jejunal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase during high-salt diet in young but not in adult rats

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Vieira-Coelho, M. Augusta, Vera A. Lucas Teixeira, Yigael Finkel, Patricio Soares-da-Silva, and Alejandro M. Bertorello. Dopamine-dependent inhibition of jejunal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase during high-salt diet in young but not in adult rats. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G1317–G1323, 1998.—During high-salt diet endogenous dopamine (DA) reduces jejunal sodium transport in young but not in adult rats. This study was designed to evaluate whether this effect is mediated, at the cellular level, by inhibition of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity. Enzyme activity was determined in isolated jejunal cells by the rate of $[^{32}P]ATP$ hydrolysis. Cells were obtained from weanling and adult rats fed either with high- or normal-salt diet. In 20-day-old but not in 40-day-old rats Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity was significantly reduced during high-salt diet. This inhibition was abolished by a blocker of DA synthesis. The decreased activity was associated with a decreased $\alpha_1$-subunit at the plasma membrane. During high-salt diet there was an increase in DA content in jejunal cells from 20-day-old rats, associated with a parallel decrease in 5-hydroxytryptamine, compared with normal-salt diet. In 40-day-old rats, however, the catecholamine level remained unchanged during high-salt diet. Incubation of isolated jejunal cells with DA resulted in a dose-dependent inhibition of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in 20- but not in 40-day-old rats. We conclude that during high-salt diet, jejunal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in 20-day-old rats is inhibited, and this effect is likely to be mediated by locally formed DA.

Sodium-potassium-adenosinetriphosphatase synthesis; serotonin; L-3,4-dihydroxyphenylalanine

WE HAVE RECENTLY SHOWN that sodium absorption and catecholamine content in the jejunal mucosa of young rats were higher than in mature animals (14). In addition, young animals during high-salt diet experienced a decrease in sodium absorption with a parallel increase in tissue levels of dopamine (DA), whereas in adult animals there were no significant changes in sodium absorption, nor in catecholamine content of the jejunal mucosa (6, 14). That study suggested that during high-salt diet, an increase in the jejunal levels of DA associated with a decrease in the norepinephrine (NE) content was responsible for the control of sodium absorption in the jejunal mucosa of young animals. Adult (40-day-old) animals did not have a significant change in jejunal sodium absorption or in catecholamines levels during high-salt diet. The lack of any change in the jejunal function in response to high-salt diet coincided with the period in which the renal function has reached maturation (23, 24), thus suggesting complementary functions between the intestine and the kidney during development.

Sodium and water homeostasis is a critical step during adaptation to early life; although nephrogenesis is completed at birth, renal tubular function continues to develop postnatally (23, 24). During early postnatal life the kidney has a limited capacity to regulate fluids and electrolyte homeostasis, leading to high sodium excretion and often to negative sodium balance and hyponatremia (30, 31). Intestinal function is also determined by a developmental process that has a great impact not only during the uptake of nutrients but, equally importantly, in maintaining electrolytes and water metabolism (17, 32). Therefore, understanding the cellular mechanisms controlling this process is of paramount importance.

In transporting epithelia, vectorial movement of sodium is accomplished by means of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase located at the basolateral plasma membrane and several sodium transport mechanisms localized at the apical domain of the cell (25). In the renal epithelia (proximal and distal tubules) of mature animals, DA decreases Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity during high-salt diet, which is believed to contribute to increased urinary sodium excretion (3). Thus the present study was designed to investigate whether DA-dependent inhibition of sodium absorption across the jejunal mucosa of young animals is associated with decreased Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity.

EXPERIMENTAL PROCEDURES

All experiments were performed in Sprague-Dawley rats (BK Universal, Sollentuna, Sweden), aged 20 (weaning) and 40 (adult) days, weighing $37.1 \pm 1.2$ g $\left(n = 8\right)$ and $170 \pm 3.4$ g $\left(n = 8\right)$, respectively. Animals were kept in air-conditioned animal quarters, in litters of four animals per litter. Adult rats were fed ordinary solid rat chow (BK Universal); young rats were given the same chow but softened by mixing it with water. Tap water was provided ad libitum. The high-salt diet group received as drinking fluid 0.9% saline instead of tap water. The 20-day-old rats on high-salt diet were given saline for 4 days, whereas the 40-day-old rats received saline for 7 days before the study. We have previously examined Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in response to high salt (3, 20) and found no differences between 2 and 7 days of administration of high-salt diet. The daily sodium intake averaged 0.5 and 5.0 mmol/100 g body wt in normal- and high-salt diet groups, respectively.
To determine the role of endogenous DA, rats were injected intraperitoneally (0.1 ml) with benserazide (10 mg/kg body wt; Roche, Basel, Switzerland), an inhibitor of aromatic l-amino acid decarboxylase (AADC), 1 h before the animals were killed. This treatment was sufficient to cause complete inhibition of the peripheral DA-converting enzyme for up to 4 h after administration (10). Control animals were injected with the same volume of vehicle (sterile water).

Cell isolation. The method for cell isolation was as described previously (18, 22) with minor modifications. Briefly, the animals were killed by decapitation under anesthesia, and the abdominal cavity was immediately opened to excise a jejunal segment ~10 cm in length. The selected segment was placed on an ice-cold glass plate, cut in smaller segments of ~1.5 cm in length, and rinsed free from blood and intestinal contents with saline (0.9% NaCl). The tissue fragments were everted with fine forceps and incubated for 45 min in 5 ml warm (37°C) and gassed (95% O2-5% CO2) Hanks’ solution (21; courtesy of Dr. M. Caplan) and the abdominal cavity was immediately opened to excise a jejunal segment and the abdominal cavity was immediately opened to excise a jejunal segment and the jejunal mucosa was removed with a scalpel. The segments of jejunum were placed in 1 ml 0.2 M perchloric acid, and stored frozen at −20°C. The assay of l-DOPA, NE, DA, and 5-hydroxytryptamine (5-HT) was performed by means of HPLC with electrochemical detection (EC) as previously described (13). Briefly, aliquots of 500 µl of perchloric acid in which the tissue had been kept were placed in 5-ml conical-based glass vials with 50 mg alumina, and the pH of the sample was immediately adjusted to 8.6 by addition of Tris buffer. Mechanical shaking for 10 min was followed by centrifugation, and the supernatant was discarded. The adsorbed catecholamines were then eluted from the alumina with 200 µl of 0.2 M perchloric acid on Spin-X microfilter tubes (Costar, Badhoevedorp, The Netherlands); 50 µl of the eluate were injected into an HPLC-EC system. The detection was performed electrochemically by means of a thin-layer cell with a glassy carbon working electrode, an Ag/AgCl reference electrode, and an amperometric detector (Gilion model 141; Gilson Medical Electronics, Villiers Le Bel, France).

The cell detector was operated at 0.75 V. The current produced was monitored using the Gilson 712 HPLC integration software connected to a personal computer system. The mobile phase was a degassed solution of 0.1 mmol/l citric acid, 0.5 mmol/l sodium octyl sulfate, 0.1 mmol/l sodium acetate, 0.17 mmol/l ethylenediaminetetraacetic acid, 1 mmol/l dibutylamine, 12% methanol (vol/vol), pH 3.5, which was pumped at a rate of 1.0 ml/min. Standard solutions of l-DOPA, NE, DA, 5-HT, and dihydroxybenzylamine (internal standard) were injected at different concentrations, and peak height increased linearly. The lower limits for detection of l-DOPA, NE, DA, and 5-HT ranged from 350 to 500 fmol.

Western blot analysis. Jejunal cells were isolated from 20- and 40-day-old rats as previously described. The cells were homogenized, and aliquots (50 µg protein) were analyzed by SDS-PAGE using the Laemmli buffer system as described (1). Proteins were transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Bedford, MA) in a buffer containing 25 mM Tris-HCl, 192 mM glycine, SDS, 0.1% (wt/vol), and 20% methanol (vol/vol). Transferring was performed at 1 Amper during 3 h using a Transphor system (Hoaffier, San Francisco, CA). Protein identification was carried out using a monoclonal antibody against the α1-subunit (21; courtesy of Dr. M. Caplan) and the α2 and α3 with a monoclonal antibody kindly provided by Dr. K. J. Sweadner.

Immunoreactivity was detected with an enhanced chemiluminescence detection kit (Amersham) used exactly as recommended by the manufacturer. Measurements were performed with multiple exposures to ensure that signals were within the linear range of the film. Scans were performed using a Scan Jet 11C scanner (Hewlett-Packard, Palo Alto, CA). Each band was scanned two times in different regions, the scans were averaged, and the area of the peak minus the background (in arbitrary units) was quantified.

Statistical analysis. The data were analyzed using Student’s t-test. Values are means ± SE. P < 0.05 was considered significant. A significant difference between one control and two experimental groups was determined using ANOVA with the Newman-Keuls posttest method (29).

The accumulation of l-DOPA in the jejunal mucosa after inhibition of AADC was calculated from a semilog plot of l-DOPA levels against time of inhibition; the slope of accumulation was calculated using linear regression. The fractional accumulation of l-DOPA (k) was then obtained from the expression k = slope/0.434 (6).

RESULTS

Na+–K+–ATPase activity was determined in isolated jejunal cells from 20- and 40-day-old rats. Basal enzyme activity, examined under optimal (maximal velocity) substrate conditions, was 300 ± 85 (n = 8) and 526 ± 51 (n = 5) nmol Pi ·mg protein− 1 ·min−1, respectively. Administration of the high-salt diet resulted in a significant decrease in Na+–K+–ATPase activity in 20- but not 40-day-old rats (Fig. 1). This decrease in 20-day-old rat Na+–K+–ATPase activity observed during high-salt diet was blunted when benserazide, an inhibitor of AADC, was given 1 h before the experiments.
The presence of different Na\(^+\)-K\(^+\)-ATPase isoforms in the jejunal cells has not been previously characterized. Thus we first determined which of the isoforms are present in jejunal cells from 20- and 40-day-old rats (Fig. 2) and whether this pattern was affected by high-salt diet. Isolated rat striatum was used as a positive control. Whereas all three isoforms were present in the striatal membranes, the jejunal cell plasma membrane exhibited the presence of only the Na\(^+\)-K\(^+\)-ATPase \(\alpha_1\)-isoform (Fig. 2). In addition, plasma membranes isolated from jejunal cells from 20-day-old rats on high-salt diet showed a significant reduction (% of control 74.2 ± 4.8, n = 4) in the \(\alpha_1\)-subunit abundance compared with age-matched controls receiving normal saline, whereas in 40-day-old animals high salt did not affect the \(\alpha_1\)-subunit abundance.

Levels of L-DOPA, NE, DA, and 5-HT were determined in the jejunal mucosa of 20- and 40-day-old rats fed normal- and high-salt diets. Basal levels of all monoamines in the jejunal mucosa of 20-day-old rats were higher than in 40-day-old rats (Table 1), in agreement with results reported in a previous study (14). In 20-day-old rats administration of high-salt diet resulted in a significant increase (~57%) in the tissue level of DA, whereas 5-HT content was significantly decreased by ~27% (Fig. 3). The tissue content of NE did not change, regardless of the diet administered. Benserazide treatment induced a significant (\(P < 0.05\)) increase in L-DOPA (from 113 ± 18.8 to 752 ± 165 pmol/g) and abolished the increase in DA during high-salt diet (Fig. 3, left) but did not induce a significant change in the tissue levels of 5-HT (Fig. 3, right). In 40-day-old rats high-salt diet did not significantly affect the levels of either DA or 5-HT (Fig. 4). During inhibition of AADC with benserazide (Fig. 5), the rate constant of accumulation of L-DOPA in the jejunal mucosa of young rats (\(k = 0.0288 ± 0.0030\), n = 6) was found to be similar (\(P = 0.09\)) to that observed in adult rats (\(k = 0.0356 ± 0.0021\), n = 7); the total amount of L-DOPA accumulated during AADC inhibition was, however, considerably higher in young rats (932 ± 39 pmol/g) than in adult rats (220 ± 33 pmol/g).

We next examined the possibility that DA may affect fluid transport across the intestinal epithelium by

### Table 1. Levels of L-DOPA, NE, DA, and 5-HT in jejunal mucosa of 20- and 40-day-old rats receiving normal-salt diet

<table>
<thead>
<tr>
<th>Condition</th>
<th>L-DOPA</th>
<th>NE</th>
<th>DA</th>
<th>5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-Day-old</td>
<td>217 ± 45*</td>
<td>755 ± 183*</td>
<td>110 ± 8*</td>
<td>9,659 ± 557*</td>
</tr>
<tr>
<td>40-Day-old</td>
<td>86 ± 29</td>
<td>35 ± 6</td>
<td>41 ± 19</td>
<td>3,440 ± 1,142</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 6–12\) animals/group. L-DOPA, \(L-3,4\text{-dihydroxyphenylalanine}\); NE, norepinephrine; DA, dopamine; 5-HT, 5-hydroxytryptamine. *Significantly different from corresponding values in 40-day-old rats (\(P < 0.05\), Newman-Keuls method).
inhibiting the Na\(^+\)-K\(^+\)-ATPase activity. In isolated cells from 20-day-old animals, in vitro incubation with DA resulted in a concentration-dependent inhibition of Na\(^+\)-K\(^+\)-ATPase activity (Fig. 6). The EC\(_{50}\) for DA-induced inhibition of Na\(^+\)-K\(^+\)-ATPase was \(\sim 100\) nM. DA (1 \(\mu\)M) failed to inhibit Na\(^+\)-K\(^+\)-ATPase activity in isolated cells from 40-day-old rats (% of control 93.1 ± 3.8, \(n = 7\), not significant).

Because the high-salt diet was not only associated with increased DA levels but also with a decrease in the cellular content of 5-HT, it was next evaluated whether the inhibitory action of DA was affected by 5-HT (Fig. 7). The inhibitory effect of DA was prevented by 1 and 10 \(\mu\)M 5-HT.

**DISCUSSION**

High-salt diet in young animals is associated with a decrease in sodium and water absorption in the small intestine (14). The results presented here suggest that at the cellular level the decreased sodium absorption (vectorial transport) could be the result of decreased Na\(^+\)-K\(^+\)-ATPase activity. Moreover, control of the Na\(^+\)-K\(^+\)-ATPase catalytic activity is likely to be dependent on endogenous DA tissue levels.

Tissue catecholamines are important regulators of jejunal cell function (7). In a previous report we have demonstrated that the ability of young animals to maintain sodium balance during high-salt diet depends on the tissue levels of DA (14). Their ability to reduce sodium and water absorption during high-salt diet is blunted by benserazide (14). The data presented here suggest that the effect of DA could be mediated by inhibition of Na\(^+\)-K\(^+\)-ATPase activity. Young rats on a high-salt diet (transport less sodium across the jejunal epithelium (14)) demonstrate lower Na\(^+\)-K\(^+\)-ATPase activity and higher DA tissue levels than young ani-
mals on normal-salt diet. The reduction in Na\(^{+}\)-K\(^{+}\)-ATPase activity during high-salt diet could be prevented by pretreatment with benzerazide, an inhibitor of DA synthesis, suggesting a causal relationship between endogenous DA and inhibition of the sodium pump. The possibility that DA may regulate Na\(^{+}\)-K\(^{+}\)-ATPase activity was further supported by experiments demonstrating that enzyme activity was reduced in jejunal cells incubated in vitro with DA and that this effect was only present in jejunal cells isolated from young but not adult rats. The finding that exogenous DA produced an effective inhibition on Na\(^{+}\)-K\(^{+}\)-ATPase activity in isolated cells from the jejunal mucosa is an additional argument favoring the view that inhibition of intestinal sodium absorption by DA may be dependent on an interaction with the enzyme.

At the cellular level inhibition of Na\(^{+}\)-K\(^{+}\)-ATPase activity by DA could be the result of a reduced number of pump units in the plasma membrane, a mechanism perhaps analogous to the one present in renal epithelial cells (8, 9). This view is supported by the finding that plasma membranes isolated from jejunal cells of young rats on a high-salt diet showed a parallel decrease in the Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha_{1}\)-subunit abundance compared with age-matched rats on a normal-salt diet.

Na\(^{+}\)-K\(^{+}\)-ATPase activity was determined under optimum substrate concentrations (sodium, potassium, and ATP), thus excluding the possibility that the inhibitory effect of DA could be secondary to changes in sodium permeability. However, the latter does not exclude the possibility that during in vivo conditions there also exists a concomitant decrease in apical sodium permeability, as reported for the renal epithelia (4, 5); a simultaneous decrease in apical sodium permeability and Na\(^{+}\)-K\(^{+}\)-ATPase activity would result in decreased vectorial transport without changes in intracellular sodium.

In contrast to data obtained from young rats, adult rats were found to show higher Na\(^{+}\)-K\(^{+}\)-ATPase activity. In addition, contrariwise to young rats, changes in neither Na\(^{+}\)-K\(^{+}\)-ATPase activity nor Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha_{1}\)-subunit abundance were found to occur in adult rats during a high-salt diet. Following the rationale used for young animals that associates sodium load, Na\(^{+}\)-K\(^{+}\)-ATPase activity, and DA levels, it might be suggested that Na\(^{+}\)-K\(^{+}\)-ATPase activity in adult rats behaved differently because jejunal cells have less ability to synthesize DA and are less sensitive to the amine; altogether, this would result in a decreased dopaminergic stimulus on the sodium pump. Several observations point in this direction. First, the basal levels of DA and L-DOPA, the amine precursor, in adult rats were one-half those in young rats. Second, exogenous DA failed to produce inhibition of Na\(^{+}\)-K\(^{+}\)-ATPase in jejunal cells of adult rats. This, however, does not explain why adult rats behave differently from young rats when challenged with a high-salt diet. Despite low levels of endogenous L-DOPA and DA in the jejunal mucosa of adult rats during normal-salt diet, the rate of L-DOPA utilization (given from the L-DOPA/DA ratios) was similar to that observed in young rats. This contrasts with the results obtained during high-salt diet, in which the rate of L-DOPA utilization in young animals was higher than in adult animals (Table 2). It appears therefore that during high-salt diet, the link between the stimulus (high-sodium intake) and the ability of the jejunal cells to increase DA synthesis is not present at an older age. However, this reduced ability of adult rats to synthesize DA may not be related to a decrease in AADC activity, because after being challenged with benzerazide the rate of accumulation of L-DOPA is similar in both groups of animals (Fig. 5, see also Ref. 6). This may indicate that the decreased ability to synthesize DA during high-salt diet is related to a reduced availability of L-DOPA to the intracellular milieu and therefore to AADC. The cellular uptake of L-DOPA, at least in renal tubular epithelial cells, has an important sodium-dependent component (26), and a possible explanation for this reduced ability of adult rats to synthesize DA could reside in the appearance of resistance to sodium-dependent mechanisms as animals become older. Although one may attribute the failure of adult animals to inhibit Na\(^{+}\)-K\(^{+}\)-ATPase activity to decreased ability to synthesize DA, it is interesting to note that in young animals the jejunal content of L-DOPA was higher than in adult animals despite the fact that they received a similar diet. On the other hand, the finding that exogenous DA is devoid of an inhibitory effect on Na\(^{+}\)-K\(^{+}\)-ATPase activity in adult rats may suggest a developmental change in the response that coincides with the maturation of the renal function and furthermore with the ability of DA to inhibit Na\(^{+}\)-K\(^{+}\)-ATPase activity in renal epithelial cells (15).

Another aspect of importance concerns the role of 5-HT, by far the most abundant amine at the level of the intestinal epithelia and a well-known intestinal secretagogue (9, 18). Although there are differences between species and between different areas of the digestive tract (jejunum, ileum, and colon), the main effect of 5-HT is a potent stimulation of intestine fluid and electrolyte secretion. This appears to result from an increase in the electrogenic secretion of chloride ions together with an inhibition of electroneutral absorption of sodium chloride (16, 33). The finding that high-salt diet in 20-day-old rats was associated with a decrease in 5-HT levels and Na\(^{+}\)-K\(^{+}\)-ATPase activity, and because in rat renal tubules 5-HT was found to stimulate the sodium pump (27, 28), it was hypothesised that at
the intestinal level an association between 5-HT levels and Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity could also be present. However, the decrease in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity during high-salt diet was abolished by benserazide, and the levels of 5-HT were not even modified. This only suggests that 5-HT, in contrast to DA, resides in a functional antagonism between the two amines in the control of enzyme activity. To our knowledge this is the first time that 5-HT in the intestine is described to inhibit Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity, and the coincubation of 5-HT agonist with DA was found to revert the inhibitory effect of the latter (27).

In the present study, the inhibitory action of maximal doses of DA on Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity was significantly prevented by coincubation with 5-HT, suggesting the presence of a functional antagonism between the two amines in the control of enzyme activity. To our knowledge this is the first time that 5-HT in the intestine is described to exert an effect on Na\textsuperscript{+}-K\textsuperscript{+}-ATPase. It is therefore difficult to integrate this information with the evidence that 5-HT decreases net sodium absorption and stimulates net chloride secretion, which necessarily needs further study.

In conclusion, the results of this study indicate that the reduced sodium absorption observed in young rats during high-salt diet is probably due to decreased Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity. This study also demonstrated that the rate of utilization of l-DOPA in jejunal cells of adults rats on high-salt diet is lower than in young rats, and this may be due to a decreased availability of the substrate to the intracellular milieu rather than a specific deficiency in AADC activity.

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