Hormonal regulation of chicken intestinal NHE and SGLT-1 activities

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De La Horra, Maria Carmen, Mercedes Cano, Maria Jose Peral, Maria Luisa Calonge, and Anunciacion Ana Ilundain. Hormonal regulation of chicken intestinal NHE and SGLT-1 activities. Am J Physiol Regulatory Integrative Comp Physiol 280: R655–R660, 2001.—The effects of aldosterone and arginine vasotocin (AVT) on intestinal Na+/H+ exchange (NHE) and Na+-sugar cotransport (SGLT-1) activities have been investigated using brush-border membrane vesicles isolated from Hubbard chicken small and large intestines, and they were compared with those induced by either Na+ depletion or dehydration. Na+ depletion was induced by feeding the chickens with either a low- or a high-Na+ diet for either 0.5, 1, 2, 4, or 8 days. Ileal and colonic NHE2 activity increased with the duration of the Na+ depletion, whereas that of intestinal SGLT-1 decreased, reaching a plateau after 2 days of treatment. Three-hour incubation of the intestine with aldosterone produced the same effects on NHE activity as does Na+ depletion, without altering SGLT-1 activity. However, 3-h incubation of the intestine with AVT increased intestinal SGLT-1 activity, without affecting intestinal NHE activity. It is concluded that aldosterone regulates apical ileal and colonic NHE2 activity, whereas that of SGLT-1 is regulated by AVT. Low-Na+ diet; aldosterone; arginine vasotocin; brush-border membrane vesicle; sodium/hydrogen exchange

IN MAMMALS, THE KIDNEY and the large intestine are the major regulators of body electrolyte and water homeostasis. As birds do not have a urinary bladder, the urine is emptied into the cloaca, and retrograde flow carries urine into the coprodeum, the colon (rectum), and cecum. As a result, the epithelia of the lower intestine process the ileal and ureteral outflow. We previously reported that dietary Na+ depletion decreased Na+-sugar cotransport (SGLT-1) activity and increased that of apical sodium/hydrogen exchange (NHE) in the chick ileum and colon (6, 7). We also showed (6) that 4 days of water deprivation increased the activities of both apical NHE and SGLT-1 in jejunum, ileum, and colon of the chicken. Therefore, Na+ depletion decreased intestinal SGLT-1 activity, whereas it was increased by dehydration. Because both treatments increased plasma aldosterone levels, it was concluded that aldosterone does not regulate intestinal SGLT-1 (6).

The purpose of the current work was to investigate in brush-border membrane vesicles (BBMV) isolated from chicken small and large intestine the time course of the effects of Na+ depletion on intestinal NHE and SGLT-1 activities and the effects of aldosterone and arginine vasotocin (AVT) on those Na+-linked transport systems activities.

MATERIALS AND METHODS

Solutions. For 22Na+ uptake experiments, the intravesicular buffer contained (in mM) 140 mannitol, 50 K-gluconate, and 50 Mes-Tris (pH 5.5). The uptake buffer consisted of (in mM) 140 mannitol, 50 K-gluconate, 0.045 valinomycin, 0.1 Na+-gluconate, tracers of 22Na+, and either 50 Mes-Tris (pH 5.5) or 50 HEPES-Tris (pH 7.5). The stop solution contained (in mM) 140 mannitol, 50 K-gluconate, 50 Mes-Tris (pH 5.5), and 0.1 amiloride. The protein concentration per assay tube was 100–150 μg/100 μl of uptake buffer.

α-Methyl-glucopyranoside (α-MG) uptake was measured in BBMV loaded with (in mM) 140 mannitol, 50 K-gluconate, 0.1 MgSO4, and 50 HEPES-Tris (pH 7.5). The extravesicular buffer contained (in mM) 140 mannitol, 50 Na-gluconate, 0.1 α-MG, 0.045 valinomycin, and tracers of α-[1-14C]MG and 50 HEPES-Tris (pH 7.5), with or without 0.25 phlorizin. The protein concentration per assay tube was 100 μg/100 μl of uptake buffer.

The everted small and large intestines were incubated for 3 h in a solution containing (in mM) 90 mannitol, 80 NaCl, 1 CaCl2, 1 MgCl2, 3 K2HPO4, 25 HCO3-choline (pH 7.4), and the desired hormone, and it was continuously bubbled with 95% O2 and 5% CO2. The medium was also supplemented with 10 mM glucose, 100 U/ml penicillin, and 100 μg/ml streptomycin. The pH of the solution was controlled throughout the experiment.

Animals and diets. Two groups of Hubbard chickens, 10 wk old, were adapted, before being killed, for either 0.5, 1, 2, 4, or 8 days to either a high-NaCl diet or a low-NaCl diet as described (14). The low-NaCl treatment consisted of low-NaCl balanced food and demineralized water containing 1 mM CaCl2. The high-NaCl treatment consisted of high-NaCl balanced food and demineralized water containing 90 mM NaCl.

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balanced food and demineralized water containing 1 mM CaCl₂ plus 0.5% NaCl (wt/vol).

Another group of chickens was fed a commercial diet for 10 wk. After chickens were killed with an intravenous injection of urethane, the small and large intestines were removed, opened longitudinally, rinsed clean with ice-cold saline solution, and incubated for 3 h in the absence or presence of either 0.5 μM aldosterone or 0.5 μM AVT.

Plasma analysis. Blood samples were collected from a wing vein into ice-cold heparinized tubes and centrifuged immediately at 4°C. Plasma aldosterone was measured by radioimmunoassay by Cerba laboratories (Barcelona, Spain). Na⁺ and K⁺ were measured by flame photometry and osmolality with an osmometer (Osmometer Gonotec, Osmomat 030). Glucose was measured enzymatically.

Glucose, Na⁺, K⁺, and osmolality of the intestinal content. The animals were killed by intravenous injection of urethane, the tissues were removed, and the content of jejunum, ileum, or colon was emptied into centrifuge tubes. After centrifugation at 5,000 rpm at 4°C for 20 min, samples were taken from the supernatant and tested for Na⁺, K⁺, glucose, and osmolality as described above.

BBMV preparation. The small and large intestines were rinsed with ice-cold saline solution and opened longitudinally. The mucose was scraped off with a glass slide, wrapped in aluminum foil, frozen in liquid nitrogen, and kept at −80°C until use.

BBMV were isolated from either jejunum or ileum as described by Peral et al. (12). Briefly, the mucose was thawed in a buffer containing (in mM) 100 mannitol, 2 HEPES-Tris (pH 7.1), 0.2 phenylmethylsulfonyl-fluoride (PMSF), 0.5 DL-dithiothreitol, and 0.2 benzamidine, homogenized in a Waring blender (model 32 BL80) at high speed for 30 s and filtered through a Buchner funnel. MgCl₂ was added to the homogenate to a final concentration of 10 mM. The suspension was stirred for 20 min and then centrifuged at 3,000 g for 15 min. The plasma membranes retained in the supernatant were collected by centrifugation at 30,000 g for 30 min. The resultant pellet was suspended in a pH 7.4 buffer consisting of (in mM) 100 mannitol, 2 HEPES-Tris, and 0.1 MgSO₄. This suspension was homogenized with 50 up-down strokes with a glass-Teflon homogenizer, brought up to 35–40 ml with the same buffer, and centrifuged at 30,000 g for 30 min. The final pellet, containing the purified BBMV, was suspended in the loading buffers as described above. The final suspension was homogenized by passing the suspension through 25- and 28-gauge needles. All the steps were carried out at 4°C.

BBMV were isolated from the colonic mucose, by the method described by Harig et al. (9), using Mg²⁺ precipitation instead of Ca²⁺ precipitation as described in Calonge et al. (3). Briefly, the mucose was defrosted in a solution (14 ml/g) containing (in mM) 50 mannitol, 0.2 PMSF, 0.5 DL-dithiothreitol, 0.2 benzamidine, and 2 HEPES-Tris, pH 7.0; homogenized with a Ystral Politron at setting 4 for 90 s, and filtered through a nylon gauze. MgCl₂ at a final concentration of 10 mM, was added to the homogenate and stirred for 20 min. The suspension was centrifuged at 1,000 g for 10 min, the resultant supernatant was centrifuged at 39,000 g for 20 min, and the resultant pellet was suspended in appropriate loading buffers. The suspension was homogenized with 20 up-down strokes with a glass-Teflon dounce homogenizer and centrifuged at 39,000 g for 30 min. The pellet was resuspended in the loading buffers described above. The isolated apical membranes were made homogenous by passing them through a 25- and a 26-gauge needle several times; they were stored in liquid nitrogen until use. All the steps were carried out at 4°C.

Protein was measured by the method of Lowry et al. (13) using albumin as the standard.

Uptake studies. ²²Na⁺ or α-[³¹C]MG uptake was measured at 25°C by a rapid filtration technique as described (4). Briefly BBMV were left to stand at room temperature (−25°C) for 10 min. Timed incubations at room temperature were initiated by adding 10 μl of suspended membrane vesicles to 90 μl of uptake buffer (100–150 μg of protein/100 μl uptake buffer). The composition of the uptake buffers is given above. After designated periods of time, uptake was terminated by the addition of 2.5 ml of an ice-cold stop solution of the same composition as that of the intravesicular buffer. The samples were immediately filtered under vacuum through a 0.45-μm pore size Millipore filter prewetted with the stop buffer. Filters were further washed twice with 5 ml of ice-cold stop solution, dissolved in 5 ml of Ready-Protein (Beckman) scintillation fluid, and radioactivity was determined by liquid scintillation spectrometry. Nonspecific isotope binding to the filters was determined separately by adding stop solution to the vesicles before addition of uptake buffer and subtracted from the total radioactivity of each sample. All experiments were done in triplicate.

Materials. ²²Na⁺ or α-[³¹C]MG were purchased from Amersham. Valinomycin, AVT, aldosterone, phlorizin, and all the salts used in the current study were obtained from Sigma Chemical. HOE-694 was a gift from Dr. M. Donowitz.

Calculations and statistics. Vertical bars in Figs. 1–5 represent SE; they are absent when they are less than symbol height. Results are expressed as means ± SE. Statistical significance was evaluated by Dunnett’s test.

RESULTS

Adaptation to a low-Na⁺ diet and plasma and intestinal fluid parameters. Plasma aldosterone levels increased nearly hyperbolically with the duration of the Na⁺ depletion from 13 to 170 pg/ml, with a half-time close to 1 day (Fig. 1). The plasma levels of either
sodium or glucose or osmolality were not affected by the treatment (Table 1).

After 12-h adaptation to a low-Na\(^+\) diet, the K\(^+\) concentration in the luminal fluid of the three intestinal regions was increased (see Table 1). The treatment also increased glucose concentration in the intestinal content, whereas that of Na\(^+\) was decreased until values were undetectable (see Table 1). Intestinal fluid osmolality did not change in the small intestine, and it was decreased in the colon (see Table 1). Although the food delivery was neither measured nor controlled, it was decreased in the colon (see Table 1). The results revealed (Fig. 2) that jejunal NHE activity was not modified by Na\(^+\) depletion, whereas both ileal and colonic NHE activity increased with the time of the duration of treatment, and the increase was due to increased NHE2 activity. NHE3 activity was not modified.

**Time-dependent effect of Na\(^+\) depletion on SGLT-1 activity in BBMV isolated from chicken intestine.** Sugar uptake into intestinal BBMV was measured in the presence of an inwardly directed electrochemical Na\(^+\) gradient, with or without phlorizin.

Feeding with a low-Na\(^+\) diet significantly decreased SGLT-1 activity in jejunum, ileum, and colon (Fig. 3). The downregulation of SGLT-1 activity reached a plateau at 2 days of treatment. Low-Na\(^+\) diet did not affect the phlorizin-insensitive sugar transport component (data not shown).

**Effect of either aldosterone or AVT on SGLT-1 and NHE activities in BBMV isolated from the jejunum, ileum, and colon.** BBMV were isolated from jejunum, ileum, and colon after 3-h incubation with or without 0.5 \(\mu\)M aldosterone or 0.5 \(\mu\)M AVT. Sugar uptake and Na\(^+\) uptake into BBMV were measured as indicated above.

The results show (Figs. 4 and 5) that aldosterone and AVT affected intestinal Na\(^+\)-linked transport systems activity differently. Thus aldosterone increased ileal and colonic NHE activity, via an increase in NHE2 activity. Jejunal NHE activity was not modified by aldosterone (data not shown). On the other hand, intestinal NHE activity was not significantly modified by AVT.

Table 1. Plasma and intestinal fluid parameters vs. the duration of NaCl depletion

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<th>0</th>
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<tr>
<td><strong>Na(^+), mM</strong></td>
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<tr>
<td>Jejunum</td>
<td>110 ± 5</td>
<td>72 ± 6(^a)</td>
<td>56 ± 8(^a)</td>
<td>46 ± 7(^a)</td>
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<tr>
<td>Ileum</td>
<td>108 ± 5</td>
<td>75 ± 4(^a)</td>
<td>45 ± 7(^a)</td>
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<tr>
<td>Colon</td>
<td>93 ± 6</td>
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<tr>
<td>Plasma</td>
<td>137 ± 4</td>
<td>130 ± 4</td>
<td>128 ± 2</td>
<td>148 ± 2</td>
<td>135 ± 3</td>
<td>137 ± 2</td>
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<td><strong>K(^+), mM</strong></td>
<td></td>
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<tr>
<td>Jejunum</td>
<td>16 ± 1</td>
<td>22 ± 1</td>
<td>29 ± 2(^a)</td>
<td>31 ± 3(^a)</td>
<td>26 ± 2(^a)</td>
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<tr>
<td>Ileum</td>
<td>14 ± 1</td>
<td>22 ± 1</td>
<td>35 ± 3(^a)</td>
<td>35 ± 3(^a)</td>
<td>38 ± 4(^a)</td>
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<td>Colon</td>
<td>30 ± 4</td>
<td>38 ± 5</td>
<td>38 ± 3</td>
<td>39 ± 6</td>
<td>32 ± 2</td>
<td>31 ± 6</td>
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<td>Plasma</td>
<td>6.3 ± 0.1</td>
<td>9.4 ± 0.7</td>
<td>8.2 ± 0.3</td>
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<td>8.7 ± 0.4</td>
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<td><strong>Glucose, mM</strong></td>
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<tr>
<td>Jejunum</td>
<td>12 ± 1</td>
<td>27 ± 2(^a)</td>
<td>41 ± 0.2(^a)</td>
<td>35 ± 1(^a)</td>
<td>41 ± 1(^a)</td>
<td>33 ± 2(^a)</td>
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<td>Ileum</td>
<td>7 ± 0.4</td>
<td>14 ± 1(^a)</td>
<td>17 ± 2(^a)</td>
<td>14 ± 1(^a)</td>
<td>13 ± 0.5(^a)</td>
<td>15 ± 1(^a)</td>
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<tr>
<td>Colon</td>
<td>1.2 ± 0.1</td>
<td>5.2 ± 0.6(^a)</td>
<td>NM</td>
<td>4.2 ± 0.6</td>
<td>6.4 ± 1(^a)</td>
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<td>Plasma</td>
<td>13 ± 0.2</td>
<td>NM</td>
<td>12.8 ± 0.3</td>
<td>13.0 ± 3</td>
<td>13 ± 0.8</td>
<td>14 ± 1</td>
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<td><strong>Osmolality, mosmol/KgH(_2)O</strong></td>
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<tr>
<td>Jejunum</td>
<td>461 ± 23</td>
<td>480 ± 14</td>
<td>482 ± 24</td>
<td>480 ± 20</td>
<td>426 ± 14</td>
<td>464 ± 26</td>
</tr>
<tr>
<td>Ileum</td>
<td>407 ± 22</td>
<td>389 ± 17</td>
<td>400 ± 14</td>
<td>418 ± 8</td>
<td>378 ± 28</td>
<td>394 ± 2</td>
</tr>
<tr>
<td>Colon</td>
<td>300 ± 15</td>
<td>230 ± 12(^a)</td>
<td>230 ± 12(^a)</td>
<td>220 ± 12(^a)</td>
<td>200 ± 12(^a)</td>
<td>200 ± 12(^a)</td>
</tr>
<tr>
<td>Plasma</td>
<td>336 ± 6</td>
<td>354 ± 28</td>
<td>336 ± 18</td>
<td>336 ± 23</td>
<td>308 ± 9</td>
<td>320 ± 7</td>
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Values are means ± SE (n = 4 animals). Glucose, Na\(^+\) and K\(^+\) concentrations, and osmolality have been measured as described in MATERIALS AND METHODS. NM, nonmeasured. --, undetectable. \(^a\)P < 0.05, \(^\ddagger\)P < 0.001 as compared with high-Na\(^+\) diet (at day 0).
Small and large intestinal SGLT-1 activity was not modified by the in vitro exposure of the tissues to aldosterone (Fig. 5), but it was stimulated by AVT.

**DISCUSSION**

The current results show for the first time that AVT stimulates intestinal SGLT-1 activity and corroborate our previous view (6) that aldosterone does not mediate the effects of Na<sup>+</sup> depletion on intestinal SGLT-1 activity.

The following observations suggest that aldosterone regulates ileal and colonic NHE2 activity, but it does not mediate the decrease in intestinal SGLT-1 activity induced by Na<sup>+</sup> depletion: 1) incubation of the intestine with aldosterone increased ileal and colonic NHE2 activity as it does Na<sup>+</sup> depletion; 2) SGLT-1 activity was decreased by Na<sup>+</sup> depletion but it was not affected by aldosterone; and 3) the time course of the effect of Na<sup>+</sup> depletion on plasma aldosterone levels is similar to that on NHE2 activity, but not to that on SGLT-1 activity.

We (6) have suggested that, because Na<sup>+</sup> depletion and dehydration increased plasma aldosterone levels and produced opposite effects on SGLT-1 activity, al-
dosterone does not regulate intestinal SGLT-1. As plasma AVT levels are increased by dehydration (1) and decreased by Na\(^+\) depletion (2), it was concluded (6) that AVT could be the regulator of intestinal SGLT-1. The current results corroborate this hypothesis, because the incubation of the intestine with AVT increased small and large intestinal SGLT-1 activity.

In our previous study (6) we also suggested that AVT regulates jejunal NHE activity because it was not affected by Na\(^+\) depletion, but it was increased by dehydration. However, the current results show that neither aldosterone nor AVT significantly affected jejunal NHE activity. These results do not agree with previous reports showing that AVT inhibits NHE3 activity in Madin-Darby canine kidney cells transfected with NHE3 (10).

It could be concluded that, in the chick, 1) apical NHE2 activity in ileum and colon is regulated by aldosterone and 2) small and large intestinal SGLT-1 activity is regulated by AVT. The current results, however, do not provide any clue to explain the increase in jejunal NHE activity induced by dehydration. It could be another hormonal factor. The decrease in intestinal SGLT-1 activity induced by Na\(^+\) depletion could be due either to a decrease in AVT secretion (2) or to the effect of Na\(^+\) content in the intestinal lumen on the expression of the SGLT-1. Thus Na\(^+\) depletion reduced Na\(^+\) concentration in the intestinal content until values were undetectable, and this decrease was accompanied by an increase in glucose concentration in the intestinal content. Further investigation is required to characterize the molecular mechanisms underlying the changes in activity of the transporters under study and to determine the link between Na\(^+\) depletion and intestinal sugar transport activity.

A collateral observation of the current study is that adaptation to a low-Na\(^+\) diet increased the K\(^+\) concentration in the luminal fluid of the three intestinal regions, indicating that the treatment stimulated intestinal potassium secretion, which agrees with previous observations in rat colon (8, 15).

**Perspectives**

The current results agree with the idea that the physiological role of the large intestinal amiloride-inhibitable Na\(^+\) absorption is to conserve NaCl and that of organic solute-Na\(^+\) cotransport is to conserve water (14) and suggest that the small intestine could also contribute to these homeostatic functions. Under Na\(^+\) depletion, both the extracellular fluid volume and...
osmolality fall (11). Regulation of extracellular fluid volume requires Na\(^{+}\) and water retention, and osmoregulation demands water excretion in a diluted urine. The increase in Na\(^{+}\)/H\(^{+}\) exchange activity and the decrease in Na\(^{+}\)-sugar cotransport activity, induced by the low-Na\(^{+}\) diet, will help to conserve Na\(^{+}\) and to excrete water, respectively. This will be achieved by increasing the plasma levels of aldosterone, which increases Na\(^{+}\)/H\(^{+}\) exchange activity, and by decreasing those of AVT, which would reduce SGLT-1 activity. On the other hand, the dehydration-induced increase in both Na\(^{+}\)/H\(^{+}\) exchange activity and Na\(^{+}\)-dependent sugar transport will help to conserve both Na\(^{+}\) and water, and, therefore, to maintain both extracellular fluid volume and osmolality within acceptable limits. From a physiological point of view, it is an advantage that the two Na\(^{+}\)-linked transport systems are regulated by a different hormone, which allows different regulation of each transporter according to the homeostatic demands of the organism. The potential physiological contribution of large intestinal active organic solute cotransport could be either to prevent the loss of sugar in the feces or to facilitate it according to the homeostatic needs of the animal.

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