Aldosterone suppresses expression of an avian colonic sodium-glucose cotransporter

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1Department of Biological Sciences, University of Delaware, Newark, Delaware 19716; 2Department of Physiology, University of Iceland, IS-101 Reykjavik, Iceland; and 3Department of Anatomy and Physiology, The Royal Veterinary and Agricultural University, DK-1870 Frederiksberg C, Denmark

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Laverty, Gary, Sesselja Bjarnadóttir, Vibeke S. Elbrønd, and Sighvatur S. Árnason. Aldosterone suppresses expression of an avian colonic sodium-glucose cotransporter. Am J Physiol Regulatory Integrative Comp Physiol 281: R1041–R1050, 2001.—Transport in the colon of the domestic fowl switches from sodium-linked hexose and amino acid cotransport on high-salt intake to amiloride-sensitive sodium channel expression on low-salt (LS) diets. The present experiments were designed to investigate the role of aldosterone in suppression of the colonic sodium-glucose luminal cotransporter (SGLT). LS-adapted hens were resalinated with or without simultaneous aldosterone treatment. Changes in the electrophysiological responses and SGLT protein expression levels were examined at 1, 3, and 7 days of treatment. Serum aldosterone levels fell from ~400 pmol/l in LS-adapted hens to values below the detection limit (~<4 pmol/l) after 1 day of resalination. At the same time, glucose-stimulated short circuit current (I_{SC}) increased from 20.9 ± 8.7 to 56.3 ± 15.5 μA/cm², whereas amiloride-sensitive I_{SC} decreased from ~68.9 ± 12.7 μA/cm² on LS to +0.6 ± 12.0 μA/cm². Glucose-stimulated I_{SC} increased further at 3 and 7 days of resalination, whereas amiloride-sensitive I_{SC} remained suppressed. When resalinated birds were simultaneously treated with aldosterone, the LS pattern of high amiloride-sensitive I_{SC} and low glucose-stimulated I_{SC} was maintained. Immunoblotting results from the same tissues demonstrated that SGLT-like protein expression increased following resalination. Aldosterone treatment completely blocked this effect. These results demonstrate that aldosterone suppresses both activity and protein expression of hen colonic SGLT. Resalination either through decreased aldosterone or other factors may be able to activate SGLT activity independently of increases in protein expression.

Birds lack a urinary bladder and instead allow for potential postrenal modification of urine by retrograde movement into segments of the lower intestine, i.e., colon and ceca (6, 26, 30, 32). Because of its role in modifying the final composition of both intestinal chyme and ureteral urine, the avian lower intestine has been referred to as the “integrating segment” (32). In the domestic fowl, the colonic epithelium is clearly adapted for high-capacity transport, with villi and microvilli amplifying the mucosal surface area (10) and evidence for a number of organic substrate and electrolyte transport pathways. These include sodium-coupled transport of hexoses and amino acids (3, 10, 24, 27) and short-chain fatty acid (22) and dipeptide (7) transport, possibly driven by \( H^+ \) ion secretion (22, 25). Organic substrates for these transport pathways may originate from either intestinal chyme or ureteral urine.

It is not clear what the actual physiological role(s) of these transport processes are, but they could serve either osmoregulatory or nutritional homeostatic functions, or both. For example, it is known that significant amounts of protein are excreted in the ureteral urine, associated with urate spheres. This protein is subsequently degraded by microbial activity in the colon and ceca (6) and likely recycled as amino acids and peptides by colonic cotransporter activities. Furthermore, recent studies with the sodium-glucose luminal cotransporter (SGLT) provide evidence for high-capacity water transport, even against osmotic gradients (28). This raises the possibility of an osmoregulatory role for similar transporters in the avian colon.

However, a puzzling aspect of this system is the repeated observation that low-salt (LS) diets reduce or even eliminate the colonic sodium-linked cotransporter activities, while concurrently inducing the expression of electrogenic amiloride-sensitive sodium channels (ENaCs) (3, 10, 27, 34). The upregulation of ENaC activity is also seen in coprodeum (the more distal segment of the avian lower intestine), and it resembles the aldosterone-mediated regulation of ENaCs in mammalian colon and other target tissues (4, 9, 15, 16, 33–35). When hens are acutely resalinated or adapted to high-salt (HS) diets, the colonic transport pattern reverts to one of sodium-linked substrate cotransport.

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with little or no amiloride-sensitive ENaC activity (3, 10, 27, 33, 34).

Although the inverse correlation between circulating aldosterone levels and dietary salt is very strong (3), it is not clear that all of the changes in the colonic transport pathways are mediated by this hormone alone (1, 10, 26, 33, 34). Aldosterone treatment, for example, has not been able to completely induce the LS pattern of transport in chronically HS-adapted hens (10, 33), although it does appear able to maintain this pattern in LS hens that are acutely resalinated (8). Recent studies on dehydrated hens actually found increased, rather than decreased, sodium-dependent colonic glucose transport, despite modestly elevated aldosterone levels (11).

Several attempts have been made recently to detect and quantify changes in SGLT-like transporter expression in the avian colon. One antibody to the rabbit intestinal SGLT-1 (21), widely used to study expression in a variety of species (29), was found to lack cross-reactivity in the hen colon, while detecting expression in the jejunum and ileum (13, 14, 20). However, a second antibody, directed against a different epitope of the same rabbit SGLT-1 (31), has been successfully used to immunodetect SGLT-like expression in the hen colon (5, 20). Studies with this antibody have shown that SGLT expression correlates directly with sodium-linked glucose transport activity and inversely with dietary NaCl (5).

Our goal in the present study was to quantify changes in both protein expression and transport activity of the hen colonic SGLT during an acute resalination/aldosterone treatment protocol in LS-adapted hens. The results demonstrate that aldosterone directly suppresses this transporter at the protein expression level.

METHODS AND MATERIALS

Animals and experimental treatments. Forty-eight White Leghorn hens (mean body wt = 1,561 ± 35 g) were divided into eight groups of six birds each for this study. Seven of these groups were adapted for at least 4 wk to a LS diet, with little or no amiloride-sensitive ENaC activity (3, 10, 27, 33, 34). Although the inverse correlation between circulating aldosterone levels and dietary salt is very strong (3), it is not clear that all of the changes in the colonic transport pathways are mediated by this hormone alone (1, 10, 26, 33, 34). Aldosterone treatment, for example, has not been able to completely induce the LS pattern of transport in chronically HS-adapted hens (10, 33), although it does appear able to maintain this pattern in LS hens that are acutely resalinated (8). Recent studies on dehydrated hens actually found increased, rather than decreased, sodium-dependent colonic glucose transport, despite modestly elevated aldosterone levels (11).

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### Table 1. Summary of experimental treatments

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet and Treatment</th>
<th>[Aldosterone]†</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td>0.02% dietary NaCl (chronic adaptation)</td>
<td>high</td>
</tr>
<tr>
<td>R1</td>
<td>LS hens/resalinated 1 day</td>
<td>low</td>
</tr>
<tr>
<td>R3</td>
<td>LS hens/resalinated 3 days</td>
<td>low</td>
</tr>
<tr>
<td>R7</td>
<td>LS hens/resalinated 7 days</td>
<td>low</td>
</tr>
<tr>
<td>RA1</td>
<td>LS hens/resalinated + aldosterone 1 day</td>
<td>high</td>
</tr>
<tr>
<td>RA3</td>
<td>LS hens/resalinated + aldosterone 3 days</td>
<td>high</td>
</tr>
<tr>
<td>RA7</td>
<td>LS hens/resalinated + aldosterone 7 days</td>
<td>high</td>
</tr>
<tr>
<td>HS</td>
<td>1% dietary NaCl + 0.5% drinking water (chronic adaptation)</td>
<td>low</td>
</tr>
</tbody>
</table>

†Aldosterone levels summarized from Table 2. LS, low salt; R, resalinated; RA, simultaneous resalination and aldosterone treatment; HS, high salt.
Agar-Ringer bridges were used to measure the transepithelial potential (PD) via paired calomel reference electrodes; Ag/AgCl wires were used to pass current. Electrical connections were made to amplified voltage clamps with solute fluid-resistance correction. The tissues were clamped to zero potential, allowing measurement of the short circuit current (I_SC), with a 1-min break in the clamp every 3 min to record the open circuit PD. Transepithelial resistance (TER) in ohms times centimeters squared is thus calculated from Ohm’s law. By convention, PD is recorded as mucosal relative to serosal side, and a positive I_SC indicates mucosal-to-serosal current flow (previously identified as net sodium flux for this tissue).

Under the conditions described above, tissues were allowed to stabilize for 60 min before beginning the experimental manipulations. At this time, glucose was added to the mucosal side to a final concentration of 20 mM, thus stimulating the sodium-coupled glucose cotransporter (SGLT). The resulting change in I_SC was monitored for 20 min. Glucose-stimulated current in this tissue has been previously shown to be blocked by phlorizin, a specific inhibitor of the SGLT (5). Subsequently, the amino acids leucine and lysine (4 mM each) were added to both sides to assess sodium-dependent amino acid transport, followed by amiloride (100 μM mucosal side only), a blocker of epithelial electrodiffusive sodium channels (ENaCs). Twenty- to thirty-minute stabilization periods were allowed between each addition.

Although currents are generally stable for several hours, we routinely calculate changes in I_SC by establishing a trend for the previous condition, extrapolating values from the last 15 min for an additional 25 min into the new treatment period. A delta I_SC was then calculated as the difference between this extrapolated pretreatment level and a stabilized posttreatment value taken 15–20 min after the addition (3).

**Immunoblotting.** A polyclonal antiserum was commercially prepared in rabbits (Quality Controlled Biochemicals, Hopkinton, MA), using a synthetic peptide corresponding to amino acids 564–575 of the rabbit intestinal SGLT (31) conjugated to keyhole limpet hemocyanin. Antibodies directed against this same epitope have been successfully used to study expression of hen colonic SGLT (5, 20). Various crude serum bleed samples were screened in our laboratory for optimal cross-reactivity against chicken colonic membrane preparations, then purified to an IgG fraction using protein A columns (Bio-Rad, Hercules, CA).

Membrane extracts were diluted and denatured in a Laemmli sample buffer containing β-mercaptoethanol to a total protein concentration of 1 mg/ml. Ten microliters (i.e., 10 μg total protein) of these extracts were then electrophoresed on an 8% SDS-PAGE minigel. Each 10-lane gel contained one sample from each of the eight groups, plus a separate lane with biotinylated molecular weight standards (Bio-Rad 161–0311). All tissue sample extracts for each bird were run twice on separate gels. The proteins were then electrotransferred overnight onto nitrocellulose membranes. The blots were blocked for 3–4 h in a Tris-buffered saline (TBS) containing 0.5% nonfat powdered milk and 0.05% Tween 20, then probed with our anti-SGLT antibody (IgG fraction diluted 1:200 in blocking solution) for 3 h at room temperature (with rocking) followed by overnight exposure at 4°C. They were then repetitively washed in TBS and TBS-Tween and exposed to horseradish peroxidase-conjugated anti-rabbit IgG (Sigma Chemical, A-0545), diluted 1:2,000, for 2 h. After a second series of washing steps, the bands were visualized using an enhanced peroxidase substrate (Opti-4CN, Bio-Rad) for 10 min. The biotinylated molecular weight standards were localized on the blots with avidin-horseradish peroxidase.

Quantitative analysis of the Western blots was performed with an Imaging Densitometer (Bio-Rad model GS-700) with associated “Molecular Analyst” software. The molecular weight of the SGLT band was determined from the quadratic regression of the molecular weight standard curve. Relative amounts of protein in the band were quantified by densitometry.

In preliminary experiments, controls were performed that included preadsorption of antibody with the antigenic SGLT peptide (20 μg/ml), dilution series with both rabbit and chicken jejunal membranes, and exposure of blots to a preimmune serum. All of these controls indicated a peptide-protectable, highly specific immunoreactive band centered at a molecular mass of 60 kDa.

**Histology and ultrastructure.** For both light microscopy (LM) and transmission electron microscopy (TEM), tissue samples were pinned to flat pieces of polystyrene and immersed in 3% glutaraldehyde in 0.1 M phosphate buffer, followed by postfixation in 1% osmium tetroxide. Tissues were dehydrated, then embedded in epon, and cut into 1-μm sections for TEM. These sections were stained with toluidine blue to highlight the different cell types present. For TEM, 40- to 70-nm ultrathin sections were cut, stained with 2% uranyl-acetate and Reynolds’s lead-citrate, and examined with a Phillips CM 100 electron microscope. Scanning electron microscopy (SEM) was also performed on colonic tissue samples. After washing and fixation in 3% glutaraldehyde/phosphate buffer, the samples were dehydrated in a graded acetone series and critical-point dried. Samples were coated with a 20-nm gold layer and examined in a JEOL 840A scanning electron microscope.

We also attempted immunohistochemical staining of colonic tissues, using both our antiserum and a sample of the purified anti-SGLT (564–575) antibody previously produced by Takata et al. (31). Both hen colon and rabbit duodenum (positive control) samples were fixed (4% buffered formalin or Bouin’s) and processed and embedded in paraffin according to conventional protocols. Immunostaining (double layered) was performed on 4- to 5-μm thick sections. Both antibody preparations localized apical brush-border staining of SGLT in rabbit duodenum, but, in all cases, staining of hen colon as well as coprodeum (negative control) was negative (see discussion).

**Analytic methods and statistics.** Plasma electrolytes were measured by flame photometry (Na⁺ and K⁺) and titration (Cl⁻) methods. Osmolarity was measured in triplicate by freezing-point depression, and aldosterone was determined with a standard radioimmunoassay with a detection limit of 44 pmol/l. Total protein was assayed using a modified Bradford reagent in a microplate format (SpectraMax 250, Molecular Devices).

All data are expressed as means ± SE. Significant differences between groups were established by ANOVA and the Tukey test for multiple comparisons.

**RESULTS**

The colonic epithelium of the domestic fowl has, in many respects, the appearance of a typical high-capacity transporting epithelium, not unlike that of the small intestine. Figures 1 and 2 present images of this tissue at both light microscopic and EM levels. The tissue is characterized by leaf-shaped villi and shallow intervillus crypts and by a simple columnar epithelium. Three major cell types are present, absorptive
epithelial cells (AEC), “dark” mitochondria-rich cells, formerly referred to as “brush” cells (MR), and mucus-secreting goblet cells (GC). In addition, occasional enteroendocrine and migrating lymphoid cells can be observed. These structural features somewhat resemble those of the coprodeum, the more distal segment of the avian lower intestine, which has been much more extensively studied (15–17). The coprodeum, however, has much flatter villi (or mucosal folds) and, of interest, demonstrates a marked shortening and loss of number of apical microvilli in hens adapted to HS diets (15, 17).

Figure 2 presents TEM images of the colonic epithelium. In Fig. 2a, structural details of the three major cell types, AEC, MR, and GC, are visible, along with the well-developed microvillus brush border. Figures 2b, c, d, and e are representative, same-magnification images of tissues from HS, LS, RA3, and RA7 hens, respectively, focusing on the microvillus brush border.
There appears to be little structural difference between HS and LS tissues, but in RA3 and RA7, there is an apparent reduction in the density and height of microvilli.

Table 2 presents the plasma electrolytes, osmolarity, and circulating aldosterone concentrations for the eight experimental groups. HS-adapted hens had plasma aldosterone levels that were below the detectable limit (44 pmol/l), whereas the LS-adapted group averaged just over 400 pmol/l. The resalination protocol used in this study reduced aldosterone levels to the detection limit within 24 h (with one R3 hen as the only exception). The aldosterone-injected groups (RA) had highly variable levels, in part, due to variation in the time between the last hormone injection and blood sampling. Nevertheless, these mean values (470–900 pmol/l) are within the range of those reported here and previously (2, 3, 9) for LS-adapted hens.

In general, acute resalination resulted in elevated plasma [Na$^{+}$], whereas aldosterone administration,

Table 2. Plasma electrolytes, osmolarity, and aldosterone concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>[Na$^{+}$], mmol/l</th>
<th>[K$^{+}$], mmol/l</th>
<th>[Cl$^{-}$], mmol/l</th>
<th>Osm, mosM</th>
<th>Aldosterone, pmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td>147.4 ± 2.5</td>
<td>4.56 ± 0.21</td>
<td>113.6 ± 0.8</td>
<td>304.3 ± 1.1;</td>
<td>404 ± 40</td>
</tr>
<tr>
<td>R1</td>
<td>167.1 ± 7.4†‡</td>
<td>3.94 ± 0.28</td>
<td>121.6 ± 3.0</td>
<td>311.4 ± 2.4;</td>
<td>&lt;44</td>
</tr>
<tr>
<td>R3</td>
<td>163.4 ± 5.0†</td>
<td>4.18 ± 0.56‡</td>
<td>119.5 ± 3.8</td>
<td>307.8 ± 3.4;</td>
<td>86 ± 42</td>
</tr>
<tr>
<td>R7</td>
<td>148.7 ± 3.1</td>
<td>3.81 ± 0.19</td>
<td>122.6 ± 1.8</td>
<td>310.8 ± 2.8;</td>
<td>&lt;44</td>
</tr>
<tr>
<td>RA1</td>
<td>160.1 ± 3.4</td>
<td>3.15 ± 0.15*</td>
<td>127.5 ± 2.0‡</td>
<td>324.5 ± 4.3‡;</td>
<td>472 ± 174</td>
</tr>
<tr>
<td>RA3</td>
<td>157.1 ± 4.3</td>
<td>3.23 ± 0.28*</td>
<td>122.7 ± 2.6</td>
<td>315.3 ± 2.0‡;</td>
<td>899 ± 511</td>
</tr>
<tr>
<td>RA7</td>
<td>162.3 ± 4.0</td>
<td>2.78 ± 0.09*</td>
<td>127.7 ± 2.6‡</td>
<td>330.8 ± 5.6</td>
<td>793 ± 339</td>
</tr>
<tr>
<td>HS</td>
<td>143.8 ± 3.9</td>
<td>3.55 ± 0.15</td>
<td>124.2 ± 2.1</td>
<td>310.6 ± 2.2‡</td>
<td>&lt;44</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 birds/group. *Values significantly different from LS group; †values significantly different from HS group; ‡values significantly different from RA7 group; §values significantly different from R3 group. Statistical differences were determined by ANOVA followed by the Tukey's multiple-comparison test, $P < 0.05$. The detection limit of the aldosterone RIA was 44 pmol/l. Osm, osmolarity.
particularly after 7 days, lowered plasma [K+] and elevated the plasma [Cl−] and osmolarity (Table 2). Of note is the fact that there were no significant differences in plasma electrolytes between the chronically adapted LS and HS groups, despite at least a 10-fold difference in steady-state circulating aldosterone concentrations. This observation presumably reflects other compensatory processes that may act to stabilize plasma volume and electrolyte concentrations.

The baseline electrophysiological data (before addition of 20 mM glucose) are presented in Table 3. Resalination with simultaneous aldosterone treatment caused, after 1 day (RA1), a large increase in baseline \( I_{SC} \) and PD, to values similar to those seen in the LS group. However, prolonged aldosterone treatment resulted in a subsequent decrease in PD (RA7), relative to LS and RA1 groups, and also decreased the TER, relative to HS or resalinated groups. TER is the only baseline parameter that was significantly different between the chronically adapted HS and LS groups.

Figure 3 shows the changes in \( I_{SC} \) from baseline, caused by addition of 20 mM glucose to the mucosal bathing solution (glucose was already present on the serosal side). Comparison of the LS and HS groups demonstrates the marked difference in physiological expression of SGLT activity. The glucose response in hens adapted to LS averaged 20.9 ± 8.7 μA/cm², whereas the response in hens on HS diets was nearly fivefold higher at 97.2 ± 11.3 μA/cm² (\( P < 0.025 \)). When LS-adapted hens were resalinated for 1, 3, or 7 days, the \( I_{SC} \) response to mucosal glucose addition showed a progressive increase, approaching the HS value by 7 days of resalination. However, parallel groups that were resalinated with simultaneous aldosterone treatment showed no difference in the \( I_{SC} \) response from the chronically adapted LS group (Fig. 3, stippled bars). These data indicate that aldosterone alone can maintain the LS pattern of low-glucose co-transport, even when superimposed on acute resalination. In this study, a small glucose-stimulated current was also observed in coprodeum from HS hens (<20 μA/cm²) but not in any of the other experimental groups.

In contrast to hexose stimulation, amino acid-stimulated \( I_{SC} \) was not significantly affected by any of the treatments. Mean values for the change in \( I_{SC} \) were 40.5 ± 8.5 and 58.0 ± 7.8 μA/cm² for the LS and HS groups, respectively, 37.4 ± 12.0, 46.5 ± 3.8, and 32.8 ± 7.6 μA/cm² for the R1, R3, and R7 groups, and 88.2 ± 37.3, 24.4 ± 10.1, and 30.3 ± 6.8 μA/cm² for RA1, RA3, and RA7 groups.

The changes in \( I_{SC} \) in response to amiloride (100 μM) are shown in Fig. 4. It can be seen that the pattern of responses is nearly opposite to that of Fig. 3, with low amiloride-sensitive current in the HS group and high sensitivity (inhibitory) in the LS group (−15.1 ± 17.2 vs. −68.9 ± 12.7 μA/cm², respectively). Even more striking is the complete suppression of amiloride sensitivity in response to resalination of LS-adapted hens (R1 group) and the marked stimulation of this sensitivity by simultaneous aldosterone treatment. Very high amiloride-sen-

### Table 3. Baseline electrophysiological properties

<table>
<thead>
<tr>
<th>Group</th>
<th>( I_{SC} ), μA/cm²</th>
<th>PD, mV</th>
<th>TER, Ω cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td>261.2 ± 41.6</td>
<td>17.0 ± 3.9</td>
<td>60.9 ± 7.9†</td>
</tr>
<tr>
<td>R1</td>
<td>116.0 ± 42.6*</td>
<td>9.0 ± 3.2</td>
<td>66.2 ± 6.4</td>
</tr>
<tr>
<td>R3</td>
<td>147.3 ± 18.6*</td>
<td>12.3 ± 1.7</td>
<td>71.9 ± 5.8</td>
</tr>
<tr>
<td>R7</td>
<td>142.8 ± 10.4*</td>
<td>11.8 ± 1.3</td>
<td>80.1 ± 6.9</td>
</tr>
<tr>
<td>RA1</td>
<td>333.2 ± 67.0</td>
<td>17.0 ± 3.9</td>
<td>48.8 ± 5.4§</td>
</tr>
<tr>
<td>RA3</td>
<td>109.0 ± 60.2*</td>
<td>6.3 ± 3.4</td>
<td>42.7 ± 6.4§</td>
</tr>
<tr>
<td>RA7</td>
<td>87.7 ± 14.9*</td>
<td>3.8 ± 1.1*</td>
<td>41.8 ± 7.0§</td>
</tr>
<tr>
<td>HS</td>
<td>101.5 ± 14.1*</td>
<td>8.7 ± 1.5</td>
<td>94.2 ± 7.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 6 \) birds/group. *Values significantly different from RA1 group; †values significantly different from LS group; ‡values significantly different from HS group; ††values significantly different from R7 group. Statistical differences were determined by ANOVA followed by Tukey’s multiple-comparison test, \( P < 0.05 \). \( I_{SC} \), short circuit current; PD, transepithelial potential; TER, transepithelial resistance.
positive currents are sustained throughout the 7 days of aldosterone treatment, whereas there is a slight rebound seen in the 3- and 7-day resalinated groups, to levels close to that of the HS group.

Colonic tissue samples taken from the same animals used in the electrophysiological studies were processed into crude membrane preparations and subjected to Western blot analysis for SGLT activity. Figure 5 shows immunoblotting results for chicken jejunum, duodenum, colon, and coprodeum, as well as a dilution series of membranes extracted from rabbit jejunum (positive control). This antiserum consistently localized a major diffuse band at 60 kDa in all tissues except coprodeum. A second nonspecific band localized at 66 kDa can also be seen in extracts from the avian tissues. The 60-kDa band, but not the 66-kDa band, was completely blockable by preadsorption of the antibody with the synthetic antigenic peptide (see METHODS AND MATERIALS).

When colonic membrane preparations from the different treatment groups (equal total protein) are assayed under identical conditions, a pattern consistent with that of glucose-stimulated $I_{SC}$ (Fig. 3) is seen. An example of a single blot with samples from all eight groups is shown in Fig. 6. There is a clear difference in the band density between the HS and LS tissue and a complete suppression of the specific 60-kDa band in all aldosterone-treated groups (RA1, RA3, and RA7). Figure 6 also shows increasing band density with time after resalination (R1-R7). Quantitative densitometry results for all tissues taken are presented in Fig. 7. Average optical density values for the HS tissues were significantly higher than those of LS ($P < 0.05$) and those of all aldosterone-treated groups ($P < 0.025$). Overall, the pattern reflects the increases in glucose-stimulated $I_{SC}$ following resalination (Fig. 3), although, on average, there is an apparent delay in the increased SGLT protein expression through the third day of resalination.

DISCUSSION

The present study confirms and expands on the findings of Bindslev et al. (5), in which protein expression levels of hen colonic SGLT were shown to be clearly correlated with dietary NaCl levels. The present results demonstrate further that aldosterone, possibly acting through one or more aldosterone-sensitive proteins, suppresses the activity and protein expression of this SGLT transporter of hen colon.

A number of earlier studies had shown that the hen colon possesses both sodium-hexose and sodium-amino acid cotransporters but that the activities of these transport processes, as measured by substrate-induced changes in $I_{SC}$, were largely suppressed or abolished in hens adapted to LS diets (3, 10, 24, 27). With decreasing dietary NaCl, there is, instead, a progressive increase in ENaC activity, correlating with a rise in circulating plasma aldosterone (3). This increase in ENaC activity has been further shown to be regulated at the mRNA level (4).

Because of the prominent changes in circulating aldosterone that accompany the variations in dietary NaCl, it may be hypothesized that aldosterone alone accounts for all of these effects, both inductive and suppressive. However, this hypothesis has been difficult to demonstrate conclusively. It is still not known, for example, whether Na$^{+}$-coupled cotransporter activity...
is upregulated by default on HS diets (i.e., absence of aldosterone) or if other factors positively affect expression under these conditions.

Similarly, it is unclear whether LS diets could suppress cotransporter activity independently of elevated aldosterone. In this regard, earlier studies with short-term aldosterone treatment of HS-adapted hens were unable to reproduce all of the effects, at least quantitatively, of LS adaptation (10, 33, 34). Furthermore, in our study of hens chronically adapted to six different dietary NaCl levels, there was a marked discrepancy in the half-maximal salt intake values for circulating aldosterone vs. amiloride-sensitive \( I_{SC} \) (3). More recently, a study of dehydrated hens demonstrated increased, rather than decreased, colonic SGLT activity, despite elevated aldosterone levels in these birds (11). Although the authors reached the conclusion that aldosterone does not regulate avian intestinal SGLT activity, it should be pointed out that aldosterone levels in the dehydrated birds were elevated to only 195 pmol/l (11), whereas LS diets can often raise aldosterone levels to 400–500 pmol/l (see Table 2 and Refs. 2, 3, 9). Moreover, not only circulating levels of aldosterone are increased by dehydration, but also those of arginine vasotocin, prolactin, and corticosterone as shown in our previous study (2). Thus it is possible, as mentioned above, that changes in other hormones could positively regulate the SGLT activity and override or minimize the effects of moderate aldosterone rises. Further studies will be needed to clarify the role of other potential regulatory factors for this system.

The discrepancy in responses between LS diets and aldosterone treatment may be explained by slow changes in target cell sensitivity or by very late effects of aldosterone, such as tissue remodeling (15–17). For this reason, we chose a protocol in which LS-adapted hens are switched to HS intake, with or without simultaneous aldosterone treatment, thus subjecting these birds to both HS “signals” and high aldosterone (1, 8). The results clearly show that aldosterone alone is able to maintain the LS transport pattern, i.e., supressed SGLT activity (Figs. 3 and 7) and high levels of amiloride-sensitive \( I_{SC} \) (Fig. 4).

These results do not rule out the possibility of other factors in contributing to the induction of SGLT and other cotransporters on HS intake. Moreover, there may be important differences among the various transport systems in their sensitivity to the effects of aldosterone. A recent similar resalination study by Arnauson (1) found, for example, partial “escape” of cotransporter activity (an increase in combined hexose and amino acid-stimulated \( I_{SC} \)) from ongoing aldosterone treatment. Clauss et al. (10) had earlier demonstrated that 24-h aldosterone treatment, given to HS-adapted hens, fully induced amiloride-sensitive \( I_{SC} \), but had no effect on amino acid-stimulated \( I_{SC} \). Furthermore, in our earlier study of hens chronically adapted to varying NaCl intake levels, it was demonstrated that galactose-stimulated \( I_{SC} \) was more sensitive to LS suppression than was amino acid-stimulated \( I_{SC} \), i.e., half-maximal suppression occurred at higher NaCl intake levels for the hexoses (3). In our current study, neither LS nor aldosterone treatment significantly affected the amino acid cotransporter activity. Thus, although some studies have demonstrated transient suppression of amino acid-stimulated \( I_{SC} \) (8, 27), this system is clearly less sensitive to the suppressing effects of aldosterone than the SGLT transporter.

We have also observed in the current study a possible lability in the morphology of the hen colon, with an apparent decrease in microvillus height and density in hens that were simultaneously resalinated and aldosterone treated (Fig. 2). In coprodeum, on the other hand, the microvillus brush border is strongly induced, along with Na\(^+\) transport activity, by aldosterone or LS diets, and it regresses on HS (16, 17). It is not clear whether aldosterone differentially affects microvillus structure in these two tissues, or if there is a transient effect associated with resalination per se. We were unable to obtain late-resalination group tissues (R3, R7) in the present study. Thus these observations are only preliminary, but clearly the possibility of tissue remodeling under various treatments deserves further study. It is notable that there appears to be little difference in microvilli structure between the LS and HS groups (Fig. 2, b and c).

In its major target tissues, the distal convoluted tubule and collecting duct of the kidney and colon, aldosterone is usually regarded as an “anabolic” hormone, upregulating both transport activity and expression of ENaC subunits, Na\(^+\)-K\(^+\)-ATPase, and other transport systems (35). However, there have been other studies implicating aldosterone in suppression of transport activities. In rat distal colon, hyperaldosteronism secondary to LS diets inhibits electroneutral NaCl absorption, thought to be mediated by parallel Na\(^+\)/H\(^+\) and Cl\(^-\)/HCO\(_3\)\(^-\) exchangers (18). In lizard colon, which is characterized as a low-resistance epithelium, multiple injections of aldosterone over a 2-day period both induced amiloride-sensitive \( I_{SC} \) and suppressed electroneutral NaCl transport, here also mediated by parallel Na\(^+\)/H\(^+\) and Cl\(^-\)/HCO\(_3\)\(^-\) exchangers (12). Interestingly, in this study, even a single injection of aldosterone 4 h before flux measurements was sufficient to induce electrogenic Na\(^+\) transport, but this acute stimulation had no effect on the electroneutral exchangers. This finding again suggests a differential sensitivity of various transport systems to aldosterone (12). More recently, Ikuma et al. (23) have demonstrated that LS diets specifically reduce the NHE2 and NHE3 isoforms of sodium-hydrogen exchanger in the rat distal colon, while increasing these same activities, including mRNA abundance, in the proximal colon.

The mechanism by which aldosterone suppresses the activity of the hen colonic SGLT is unknown at this time. However, both the present study and that of Bindslev et al. (5) indicate that LS diets and aldosterone treatment reduce the protein expression level of SGLT in this tissue. This is supported by kinetic studies demonstrating a reduced \( V_{\text{max}} \) but unchanged \( K_m \) for glucose uptake in brush-border membrane vesicles from LS- vs. HS-adapted hens (13, 19). The reduction
in transporter expression on LS diets would also imply an increased synthesis of SGLT in hens undergoing resalination. However, it is also possible that aldosterone might independently inactivate a basal pool of SGLT transporters through the actions of regulatory proteins or by removal from the plasma membrane. This could account for the apparent delay in increased SGLT protein expression during our resalination protocol (between 3 and 7 days, see Fig. 7), even while Na⁺-glucose cotransporter activity rises after only 1 day of resalination (Fig. 3). Thus, during the early stages of resalination, basal SGLT activity may be reactivated or reinserted into the apical microvillus membrane, whereas induction of new SGLT protein would require a longer period of treatment.

There have been several recent studies attempting to use immunologic approaches to quantify changes in hen colonic SGLT. An antibody prepared against the rabbit intestinal SGLT-1 amino acid sequence 402–420 (21), which has been widely used in comparative studies (29), was shown to react with hen jejunal and ileal SGLT, but not to the colonic SGLT (13, 14, 20). However, a second antibody prepared against amino acid sequence 564–575 of the same rabbit intestinal SGLT, but not to the colonic SGLT (13, 14, 20). Howes (29), was shown to react with hen jejunal and ileal (21), which has been widely used in comparative studies (29), was shown to react with hen jejunal and ileal SGLT, but not to the colonic SGLT. An antibody prepared against the rabbit intestinal SGLT-1 amino acid sequence 402–420 hen colonic SGLT. An antibody prepared against the tertiary structure or position of the protein in the consensus across species, there may be differences in these colonic transport systems to address questions of physiological function of this system, however, is still unclear.

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