Aldosterone increases oxygen consumption of rectal epithelia of normal, sodium-deprived and sodium-loaded rats

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Summary

Introduction. The colonic epithelium is a classical aldosterone target, but the effect of the hormone on the oxygen consumption rate (\(QO_2\)) of this tissue is unknown. Objectives. We aimed at assessing, in the rectal epithelium of rats fed with diets of different sodium content, the effect of epithelial sodium channel (ENaC) blockade on short-circuit current (\(I_{SC}\)) and \(QO_2\), and the acute effect of aldosterone incubation on ISC and \(QO_2\). Methods. Adult male rats were fed with normal, low or high-sodium diets for 8 days. Plasma sodium and serum aldosterone were measured. Isolated mucosa preparations from the rectal portion of the colon were mounted in Ussing chambers modified to measure \(I_{SC}\) and \(QO_2\). Results. Baseline \(I_{SC}\) and \(QO_2\) were highest in sodium-deprived rats. Both were proportionally reduced by amiloride (0.1 mM) in this group and in the normal sodium group, but not in sodium-loaded rats. Plasma sodium and serum aldosterone were measured. Isolated mucosa preparations from the rectal portion of the colon were mounted in Ussing chambers modified to measure \(I_{SC}\) and \(QO_2\). Results. Baseline \(I_{SC}\) and \(QO_2\) were highest in sodium-deprived rats. Both were proportionally reduced by amiloride (0.1 mM) in this group and in the normal sodium group, but not in sodium-loaded rats. In separate experiments, incubation with aldosterone (10 nM) for 7 h increased \(I_{SC}\) and \(QO_2\) in all groups; increases were larger in the normal and sodium-loaded groups. Amiloride decreased both \(I_{SC}\) and \(QO_2\), abolishing the differences between groups. Linear regression of the decrease in \(QO_2\) and \(I_{SC}\) after amiloride showed the steepest slope for the sodium-deprived group and the flattest one for the sodium-loaded group. Conclusions. Baseline epithelial \(QO_2\) of sodium-deprived and control rats is reduced by ENaC blockade. Aldosterone increased \(QO_2\) proportionally to \(I_{SC}\) augmentation in all groups, but the coupling between aerobic metabolism and electrogenic transport seems more efficient in sodium-deprived animals.

Key words. Aldosterone, ENaC, oxygen consumption, rat rectal colon, short-circuit current, sodium intake.

La aldosterona aumenta el consumo de oxígeno del epitelio rectal de ratas normales, privadas de sodio y cargadas de sodio

Resumen

Introducción. El colon es un blanco clásico de la aldosterona, pero su efecto sobre el consumo de oxígeno (\(QO_2\)) del epitelio se desconoce. Objetivos. Evaluar el efecto del bloqueo de canales epiteliales de sodio (ENaC) sobre la corriente de cortocircuito (\(I_{SC}\)) y el \(QO_2\) del epitelio rectal de ratas con diferentes ingestas de sodio, y el efecto agudo de la incubación con aldosterona sobre \(I_{SC}\) y \(QO_2\). Métodos. Ratas machos adultos recibieron dietas de contenido bajo, normal o elevado de sodio por 8 días. Se midió sodio plasmático y aldosterona sérica. Se montaron preparaciones de mucosa aislada en una cámara de Ussing modificada para medir \(I_{SC}\) y \(QO_2\). Resultados. Baseline \(I_{SC}\) y \(QO_2\) fueron máximos en las ratas privadas de sodio. Ambos se redujeron de manera proporcional por amiloride (0.1 mM) en el grupo de sodio privado y en el grupo de sodio normal, pero no en el grupo de sodio elevado. Se midieron el sodio plasmático y la aldosterona sérica. Se montaron preparaciones de mucosa aislada en una cámara de Ussing modificada para medir \(I_{SC}\) y \(QO_2\). Resultados. Baseline \(I_{SC}\) y \(QO_2\) fueron máximos en las ratas privadas de sodio. Ambos se redujeron de manera proporcional por amiloride (0.1 mM) en el grupo de sodio privado y en el grupo de sodio normal, pero no en el grupo de sodio elevado. En experimentos separados, la incubación con aldosterona (10 nmol/L) por 7 h aumentó \(I_{SC}\) y \(QO_2\) en los tres grupos; los aumentos fueron mayores en las ratas normales y cargadas de sodio. La amilorida redujo \(I_{SC}\) y \(QO_2\), y en los tres grupos; los aumentos fueron mayores en las ratas normales y cargadas de sodio. La amilorida redujo \(I_{SC}\) y \(QO_2\), aparentando las diferencias entre grupos. La disminución de \(QO_2\) y \(I_{SC}\) luego de amilorida tuvo una pendiente máxima de regresión en...
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Two major functions of the colon are dehydration of luminal content and regulation of fecal electrolyte excretion. Net ion absorption provides the driving force for water absorption and is dependent on aerobic metabolism. Acute hypoxia reduces net ionic transfer. In the rat distal colon, decreases in chloride secretion are associated with reductions in oxygen consumption ($O_2$), while stimulated chloride secretion is associated with increased $O_2$.

We have previously described the relationship between chloride secretion and $O_2$ in rat distal colon under basal and stimulated conditions and between electrogenic sodium absorption and $O_2$ in the human colon.

Under normal conditions, electrogenic transport in distal human colon reflects mostly sodium absorption, while in rats it is due to chloride secretion, with sodium absorption occurring through an electroneutral, coupled NaCl process. However, like other ion transporting epithelia, the colonic epithelium is a classic target tissue for the action of mineralocorticoids.

In the rat distal colon, elevated mineralocorticoid levels switch the major sodium absorption mechanism from electroneutral NaCl absorption to electrogenic sodium absorption. This change may be caused by sodium deprivation, which increases aldosterone secretion, by administration of mineralocorticoids in vitro or by exposure of the epithelium to aldosterone in vitro. The effect of incubation with aldosterone is maximal in the latest portion of the distal colon, also called rectal colon, or simply rectum.

Sodium deprivation increases expression of both β and γ subunits of the epithelial sodium channel (ENaC) in rat distal colon. Interestingly, rat rectal epithelium shows amiloride-sensitive sodium transport even in rats fed on a normal sodium diet, and its magnitude is increased by incubation with aldosterone. It is not clear whether changes in sodium intake that have an effect on endogenous aldosterone secretion can modify the epithelial response to exogenous aldosterone, and specifically if this response is depressed during sodium overload. However, it is known that ENaC function is disturbed in inflammatory bowel diseases, accounting in part for its diarrheal manifestation, which is mostly due to impaired sodium and water absorption. For example, in patients with Crohn’s disease, sodium absorption in non-inflamed portions of the sigmoid colon is impaired due to a reduced expression of the ENaC γ subunit. Furthermore, in the colonic mucosa from patients with ulcerative colitis, aldosterone-stimulated sodium absorption in the sigmoid colon is strongly inhibited. This is associated with reduced expression of ENaC β and γ subunits, apparently caused by tumor necrosis factor alpha.

We are not aware of reports on whether aldosterone-induced increases in amiloride-sensitive electrogenic transport modify epithelial $O_2$. In the present study, we assessed the effects of aldosterone incubation on short-circuit current ($I_{sc}$) and $O_2$ in rectal epithelia from rats submitted to control, low sodium and high sodium diets.

Material and methods

Ethics

The experimental protocol was designed according to the National Institutes of Health (USA) guidelines for animal research. It was reviewed and approved by the Committee for Animal Care and Biosafety of our Medical School.

Animals

Adult Wistar-Hokkaido male rats weighing 250 to 300 g were used. They were brought from the Medical School Animal Facility and housed at 24 ºC, one animal per cage, with a 12/12 h light/dark cycle. Animals were randomly assigned to be fed for 8 days with a standard diet, a low-sodium diet or a high-sodium diet (n = 18 for each group). All rats ate food and drank fluids ad libitum.

Diets

The control group was fed with a standard pelleted diet with a sodium content of 220 μmol/g (Cargill Co). The low-sodium group was given a diet with a sodium content of 0.7 μmol/g (pelleted Sodium Deficient Diet, # 902902, ICN Biomedicals, Inc). The potassium content of both diets was 300 μmol/g. For the control group and the sodium-deprived group, the drinking fluid was dis-

Abreviaturas

$O_2$: oxygen consumption rate.
ENaC: epithelial sodium channel.
$I_{sc}$: short-circuit current.
$R_{TE}$: transepithelial resistivity.

Palabras claves. Aldosterona, colon rectal de rata, consumo de oxígeno, corriente de cortocircuito, ENaC, ingesta de sodio.

rats privadas de sodio y mínima en ratas cargadas de sodio.

Conclusiones. El $O_2$ basal de ratas normales y privadas de sodio disminuye por bloqueo de ENaC. La aldosterona incrementó proporcionalmente $O_2$ e $I_{sc}$ en todos los grupos, pero el acoplamiento entre metabolismo aeróbico y transporte electrogénico parece más eficiente en los animales privados de sodio.

Aldosterona, colon rectal de rata, consumo de oxígeno, corriente de cortocircuito, ENaC, ingesta de sodio.
tilled water. The high-sodium group received the same diet than the control group, but the drinking fluid was saline (0.9 g/dl sodium chloride; 154 μmol of sodium per milliliter). For each animal, food and fluid consumption were estimated by weighing the remaining pellets and drinking fluid each morning. These data were used to estimate daily sodium intake.

Gas, solution and drugs
A mixture of 95% O₂ and 5 % CO₂ (Air Liquide, Inc) was used. The Ringer solution had the following composition: 132.8 mM Na; 4.5 mM K; 1.25 mM Ca; 1.0 mM Mg; 114 mM Cl; 24 mM HCO₃; 0.8 mM HPO₄; 0.2 mM H₂PO₄; 10 mM D-glucose; 0.5 mM β-hydroxybutyrate; 2.5 mM glutamine and 10 mM D-mannose. When gassed to saturation, the pH of the solution was 7.40. Aldosterone and amiloride were purchased from Sigma-Aldrich. Gentamicin (Schering-Plough) was added to the Ringer solution for a final concentration of 91 µg/mL to prevent bacterial overgrowth. Aldosterone was dissolved in absolute ethanol and amiloride in dimethyl sulfoxide to yield final chamber concentrations of 10 nmol/L and 0.1 mmol/L, respectively. At the added volumes, neither ethanol (10 µL) nor dimethyl sulfoxide (25 µL) had any effect on Iₑ or QO₂.

Surgery, dissection and mounting
Under ether anesthesia, rats were killed by thoracotomy. The abdomen and the pelvic bones were cut open to remove the whole colon, from the cecum to the anus. Segments from the pelvic portion of the colon, beyond a lymph node located at the pelvic brim – also called “late distal colon” or “rectal colon”– were used. The microdissection technique has been described before. Isolated mucosa preparations were obtained, cut open, gently stretched, and mounted as flat sheets in Ussing chambers.

Ussing chamber
The modified Ussing chamber used in the present experiments has been previously described. It was airtight and had an opening of 1 cm². Each hemichamber had a bubble trap through which drugs may be injected, and a port for inserting a polarimetric oxygen probe (CellOx 325) connected to WTW Oxi 340 oxygen meter (WTW GmbH). The probes allowed continuous measurement of oxygen concentration and temperature. Each hemichamber had a small magnetic bar in its bottom for continuous mixing of contents when placed on a magnetic stirrer (HI 300N, Hannah Instruments). The temperature was kept at 37.0 ± 0.5 °C by an inbuilt water jacket.

Oxygen consumption and short-circuit current determination
Oxygen meters were calibrated according to the user’s manual. Their slopes were checked before each experiment. QO₂ was calculated from the rate of change in oxygen concentration, chamber volume and oxygen solubility at 37 °C. Blankruns were performed after each experiment, to make sure that the rate of decrease in oxygen concentration was below 5% of baseline epithelial QO₂.

Calomel electrodes were connected to each hemichamber through 3% agar-in Ringer bridges to record transepithelial potential difference. An amplifier, with correction for bridge asymmetry and solution resistivity, allowed passing current through Ag/AgCl electrodes for clamping the transepithelial potential difference at 0 mV. Experiments were performed under the short-circuit condition, except for brief releases to measure open circuit potential difference. Transepithelial resistivity (Rₑ) was calculated according to Ohm’s law.

Plasma sodium and serum aldosterone determination
Trunk blood was withdrawn during surgery. An aliquot was treated with calcium heparine (Calciparine) and centrifuged at 1000 rpm for 30 min. Sodium concentration was measured with an 84-11 Orion Ross sodium electrode connected to a 720 A meter (Orion Research, Inc). The remaining blood was allowed to clot. Its serum was frozen for later determination of aldosterone concentration with a coated-tube radioimmunoassay (Diagnostic Products Corporation).

Experimental procedures
In a first set of experiments, the response of epithelial samples of the three groups to amiloride was assessed. The chamber was filled with Ringer, gassed to saturation, and closed. A 30-min period was allowed for stabilization of Iₑ, after which baseline QO₂ was measured for 30 min. Amiloride was then added and, after Iₑ plateaued at a lower level, a second QO₂ determination was performed.

A second set of experiments evaluated the response to incubation with aldosterone. The starting procedure was the same as that just described. However, after the first QO₂ measurement, aldosterone was added to both hemichambers. After 7 h, QO₂ was measured by a second 30-min period. Then amiloride was added to the mucosal hemichamber and 20 min later a third QO₂ measurement was performed (Figure 1).

During the aldosterone incubation period, the chamber was twice opened, gassed and closed again, to avoid excessive decreases in oxygen concentration at the end of the experiment, when two additional QO₂ measurements
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Comparison Test, was employed for comparing the data from the three groups. ANOVA for repeated measures was used for comparing the effect of treatments within each group. Kolmogorov-Smirnov normality test was performed to check for deviations from a Gaussian distribution. Linear regression analysis, with a check for significant deviation from linearity, was used for assessment of the relationship between $I_{SC}$ and $QO_2$. Unless otherwise stated, results are expressed as mean ± SEM. Differences were deemed statistically significant at $p < 0.05$.

Results

Food, fluid and sodium intake

Data on food, fluid and calculated sodium intake are shown in Table 1. There was no significant difference in food intake. However, fluid intake was highest in sodium-loaded animals and lowest in sodium-deprived animals. Daily sodium intake of sodium-deprived rats (32.8 ± 0.6 mg/kg of body weight) was about 3% of sodium intake of control rats (1031.0 ± 19.2 mg/kg), while it was 214% of the latter in sodium-loaded rats (2204 ± 38.0 mg/kg).

Table 1. Daily food, fluid and sodium intake of the three groups of rats.

<table>
<thead>
<tr>
<th></th>
<th>Food intake (g/kg b.w.)</th>
<th>Fluid intake (ml/kg b.w.)</th>
<th>Sodium intake (mmol/kg b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal sodium ($n = 18$)</td>
<td>80.1 ± 1.5</td>
<td>120.3 ± 2.2</td>
<td>17.62 ± 0.33</td>
</tr>
<tr>
<td>Sodium-deprived ($n = 18$)</td>
<td>80.0 ± 0.9</td>
<td>110.6 ± 2.5</td>
<td>0.56 ± 0.01</td>
</tr>
<tr>
<td>Sodium-loaded ($n = 18$)</td>
<td>79.7 ± 1.4</td>
<td>131.2 ± 2.4</td>
<td>37.86 ± 0.6</td>
</tr>
</tbody>
</table>

*Values are mean ± SEM. *$p = 0.978$ by ANOVA. No additional test was performed. **$p < 0.0001$ by ANOVA. Differences between all three groups were significant ($p < 0.05$) by Tukey’s Multiple Comparison Test.

Plasma sodium and serum aldosterone

The results of these measurements are shown in Table 2. Serum sodium was higher in sodium-loaded animals than in both the control and the sodium-deprived group. Although the mean value of the sodium-deprived group was lower than that of the control group, the difference was not significant. Serum levels of aldosterone were markedly different, with the sodium loaded group having about a five times lower concentration, and the sodium-deprived group having a more than five-fold higher concentration than the control group.
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**Effect of amiloride**

The effects of amiloride on non-stimulated epithelial samples from the three groups are shown in Table 3. Amiloride induced significant changes in I_{SC}, R_{TE}, and QO₂ in both the control and the sodium-deprived groups. Baseline I_{SC} was highest in sodium-deprived rats, intermediate in control rats and lowest in sodium-loaded rats (p < 0.0001), although the difference between the two latter groups was not significant. After amiloride, the I_{SC} of the sodium-loaded group was higher than those of the other two groups (p < 0.0001), which did not significantly differ between them.

Table 3. Effects of amiloride on short-circuit current, transepithelial resistivity and oxygen consumption of rat rectal epithelium.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Amiloride***</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-circuit current (µEq/h/cm²)**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.56 ± 0.14</td>
<td>0.97 ± 0.11</td>
<td>0.0002</td>
</tr>
<tr>
<td>Sodium-deprived</td>
<td>6.74 ± 0.32</td>
<td>0.82 ± 0.08</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Sodium-loaded</td>
<td>1.70 ± 0.12</td>
<td>1.65 ± 0.13</td>
<td>0.5577</td>
</tr>
<tr>
<td>Transepithelial resistivity (Ω/cm²)**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>77.7 ± 3.4</td>
<td>84.7 ± 3.2</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Sodium-deprived</td>
<td>68.5 ± 2.8</td>
<td>81.5 ± 2.3</td>
<td>0.0003</td>
</tr>
<tr>
<td>Sodium-loaded</td>
<td>88.2 ± 3.4</td>
<td>90.2 ± 2.8</td>
<td>0.1672</td>
</tr>
<tr>
<td>Oxygen consumption (µmol/h/cm²)**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.87 ± 0.08</td>
<td>2.44 ± 0.09</td>
<td>0.0012</td>
</tr>
<tr>
<td>Sodium-deprived</td>
<td>3.12 ± 0.10</td>
<td>2.21 ± 0.08</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Sodium-loaded</td>
<td>2.75 ± 0.12</td>
<td>2.70 ± 0.16</td>
<td>0.5567</td>
</tr>
</tbody>
</table>

* Values are mean ± SEM. ** p = 0.0003 by ANOVA. Plasma sodium was significantly higher (p < 0.05) in the sodium loaded group than in the other two, which did not differ between them according to Tukey’s Multiple Comparison Test. *** p < 0.0001 by ANOVA. The mean of each group is significantly different from that of the others (p < 0.05) by Tukey’s Multiple Comparison Test.

Baseline R_{TE} was significantly different (p = 0.0025), with a lower value in tissues from sodium-deprived rats and a non-significant differences between the control group and the sodium-loaded one. After amiloride, R_{TE} was not different between groups (p = 0.1192). Also, baseline QO₂ was not significantly different between groups (p = 0.0870), although there was a significant linear trend (p < 0.05) for increasing values from sodium-loaded, to control to sodium-deficient rats. After amiloride, values of QO₂ were significantly different (p = 0.030) because sodium-deprived QO₂ was significantly lower than sodium-loaded QO₂, while control QO₂ did not differ significantly from either.

While the difference in mean values of I_{SC}, R_{TE} and QO₂ before amiloride and after it was not always significant, the changes in all three variables induced by amiloride were highly significant (p < 0.0001), with values for each group which were significantly different from the other two groups (Table 3).

**Incubation with aldosterone**

Data for I_{SC} and QO₂ under baseline condition, after a 7-h incubation with aldosterone and after addition of amiloride are shown in Table 4. As observed in the first set of experiments, baseline I_{SC} was higher in samples from sodium-deprived rats than in samples from control rats or sodium-loaded rats, but the latter two groups did not differ between them in either I_{SC} or QO₂ at the baseline measurement. At the end of the aldosterone exposure period, both I_{SC} and QO₂ were significantly raised in all three groups. However, the absolute and fractional increases in both variables were larger in the control and sodium-loaded groups. Amiloride addition abolished all differences in I_{SC} (p = 0.6982) and QO₂ (p = 0.8979).

The decreases in I_{SC} and QO₂ recorded after adding amiloride to the mucosal side of the epithelia were significantly correlated in all three groups (all p < 0.0001; Figure 2). No significant deviation from linearity was found in any group. However, the slope of each line was significantly different from the other two (p < 0.0001). The line was steepest for samples from sodium-deprived rats, intermediate for samples from control rats and least steep in samples from sodium loaded rats. Thus, after amiloride addition at the end of the aldosterone incubation period, the mean absolute change in I_{SC} per unit of absolute change in QO₂ was 1.90 µEq/h/cm² (51 µA/cm²) per µmol/h/cm² for samples from sodium-loaded rats, 3.56 µEq/h/cm² (95 µA/cm²) per µmol/h/cm² for samples from control rats and 4.58 µEq/h/cm² (123 µA/cm²) per µmol/h/cm² for samples from sodium-deprived rats.

Table 2. Plasma sodium and serum aldosterone at the time of death of the animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma sodium (mmol/L)*</th>
<th>Serum aldosterone (nmol/L)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal sodium (n = 18)</td>
<td>141.9 ± 1.2</td>
<td>1.47 ± 0.06</td>
</tr>
<tr>
<td>Sodium-deprived (n = 18)</td>
<td>138.3 ± 1.1</td>
<td>8.06 ± 0.22</td>
</tr>
<tr>
<td>Sodium-loaded (n = 18)</td>
<td>145.2 ± 1.0</td>
<td>0.30 ± 0.05</td>
</tr>
</tbody>
</table>

* Values are mean ± SEM. * p = 0.0003 by ANOVA. Plasma sodium was significantly higher (p < 0.05) in the sodium loaded group than in the other two, which did not differ between them according to Tukey’s Multiple Comparison Test. ** p < 0.0001 by ANOVA. The mean of each group is significantly different from that of the others (p < 0.05) by Tukey’s Multiple Comparison Test.
Discussion

We have previously estimated the fraction of epithelial oxygen consumption associated with electrogenic sodium transport in the non-stimulated human colon. As far as we know, this is the first study reporting the change in oxygen consumption associated with aldosterone-stimulated sodium transport of the rectal epithelium. The three groups of rats differed markedly in calculated sodium intake. Correspondingly, compared to the standard diet, the low-sodium and high sodium diets were able to significantly increase and decrease, respectively, plasma aldosterone levels. Only the terminal portion of the colon was employed in our experiments, since more proximal portions of the rat distal colon do not show such large increases in sodium-dependent short-circuit current upon incubation with physiological aldosterone concentrations.

Table 4. Short-circuit current and oxygen consumption rate at baseline, after incubation with aldosterone and after exposure to amiloride.

<table>
<thead>
<tr>
<th></th>
<th>Baseline*</th>
<th>Aldosterone*</th>
<th>Amiloride*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(μEq/h/cm²)</td>
<td>(μEq/h/cm²)</td>
<td>(μEq/h/cm²)</td>
</tr>
<tr>
<td>Controls*</td>
<td>2.83±0.10</td>
<td>2.64±0.08</td>
<td>7.93±0.19</td>
</tr>
<tr>
<td>Sodium-deprived*</td>
<td>7.44±0.20</td>
<td>3.26±0.12</td>
<td>9.10±0.24</td>
</tr>
<tr>
<td>Sodium-loaded*</td>
<td>2.76±0.09</td>
<td>2.67±0.07</td>
<td>7.95±0.16</td>
</tr>
</tbody>
</table>

*Values are mean ± SEM. Differences between groups (columns) were analyzed by ANOVA followed by Tukey’s multiple comparison test. **n = 12 for each group. Changes within each group (rows) were analyzed by repeated measures ANOVA followed by Tukey’s multiple comparison test. *I_SC = short-circuit current; QO₂ = oxygen consumption rate. † For both I_SC and QO₂, ANOVAs p < 0.0001, with the mean of baseline and both treatments being significantly different between them at p < 0.05. **ANOVA’s p < 0.0001; Sodium-deprived group significantly different (p < 0.05) from the other two groups. ***ANOVA’s p = 0.0002; Sodium-deprived group significantly different (p < 0.05) from the other two groups. ****ANOVA’s p = 0.0101. Sodium-deprived group significantly different (p < 0.05) from the other two groups.

Figura 2. Regression analysis of the change in short circuit current (I_SC) and oxygen consumption rate (QO₂) after addition of amiloride (0.1 mM) in rectal epithelial samples incubated for 7 h with aldosterone. p < 0.0001 for all groups. Coefficients of determination (r²) were r² = 0.963 for controls, r² = 0.934 for sodium-deprived rats and r² = 0.941 for sodium-loaded rats.

Discussion

We have previously estimated the fraction of epithelial oxygen consumption associated with electrogenic sodium transport in the non-stimulated human colon. As far as we know, this is the first study reporting the change in oxygen consumption associated with aldosterone-stimulated sodium transport of the rectal epithelium. The three groups of rats differed markedly in calculated sodium intake. Correspondingly, compared to the standard diet, the low-sodium and high sodium diets were able to significantly increase and decrease, respectively, plasma aldosterone levels. Only the terminal portion of the colon was employed in our experiments, since more proximal portions of the rat distal colon do not show such large increases in sodium-dependent short-circuit current upon incubation with physiological aldosterone concentrations.
Epithelial samples from control rats and sodium-loaded rats did not differ in baseline $QO_2$ or $I_{sc}$, although plasma aldosterone of sodium loaded rats was only about 20% of that of control rats. However, in the rectal epithelium of control rats, fed with a normal sodium diet, an amiloride-sensitive fraction of baseline $I_{sc}$ was measured, as previously reported by others. This component of $I_{sc}$ was absent in tissues from sodium-loaded rats. Considering the plasmatic concentration of aldosterone of each group (Table 2), these findings agree with a report stating that the threshold for an aldosterone effect in vitro -- in the absence of plasmatic binding proteins -- is 0.3 nM.

On the other hand, epithelial samples from sodium-deprived rats had a higher baseline $I_{sc}$ than the other two groups. This current was sensitive to amiloride 0.1 mM, indicating electrogenic sodium transport. Aldosterone was present in the bath throughout the incubation period, since it has been shown that the stimulating effect of aldosterone in vitro fades if the hormone is removed from the bathing solution.

An aldosterone concentration of 3 nM has been found quite effective to induce electrogenic transport in the rat rectal colon in vitro. However, for the experiments reported here we chose to incubate the tissues with aldosterone at 10 nM (as Inagaki et al. did), a concentration that still is within the range of physiological response.

One reason for this modification was that preliminary experiments showed that the response to incubation with 3 nM aldosterone was quite variable under our experimental conditions. A second and more important reason was that we wished to assess, in sodium-deprived rats, whether exposure to an aldosterone level above their plasma aldosterone concentration resulted in further stimulation of electrogenic sodium transport. This assessment could be done because plasma aldosterone in sodium-deprived rats was below 10 nM, also taking into account that free aldosterone is between 30% and 50% of total plasma concentration. Thus, epithelial samples from sodium-deprived rats were exposed to an aldosterone concentration up to three times higher than their free plasma level.

In fact, incubation with aldosterone significantly increased $QO_2$ and amiloride-sensitive $I_{sc}$ in all three groups. The changes were least dramatic in the sodium-deprived group, whose values of both short-circuit current and $QO_2$ were already higher at baseline than those of the control and sodium-loaded groups.

As it could have been expected from previous reports, amiloride drastically decreased $I_{sc}$ and $QO_2$ after incubation with aldosterone. In this case, the decreases were larger in the sodium-deprived group. As a result, after amiloride addition at the end of the aldosterone incubation period, there was no significant difference in either $I_{sc}$ or $QO_2$ between samples from the three groups.

In all groups there was a highly significant linear correlation between the reductions in $I_{sc}$ and $QO_2$. We have previously shown that there are significant linear correlations between chloride secretion and $I_{sc}$ in the rat distal colonic epithelium. We have also found a significant linear relationship between short-circuit current and $QO_2$ in the unstimulated human sigmoid colon.

Present results indicate that qualitatively similar effects are also observed in tissues from both sodium-loaded and sodium-deprived animals.

However, finding significantly different slopes for each experimental group of the relationship between changes in $QO_2$ and short-circuit current was an unexpected result. Currently, we do not have an obvious explanation for this fact, which deserves further research. The different slopes indicate that, in this epithelium, the ratio between net sodium absorption and $QO_2$ is not fixed. The metabolic cost of sodium transport was lowest in tissues from sodium-deprived animals and highest in tissues from sodium-loaded ones. This suggests that sodium deprivation induces an improvement in the efficacy of ion transport, while dietary sodium excess has the opposite effect. It is tempting to speculate that this is related to the different, chronically sustained, plasmatic aldosterone levels of each group. The relatively fast effect reported is totally or mostly non-genomic, but the differential effect for each group might depend on some complex interaction or "cross talk" between genomic and non-genomic pathways.

In the human distal colon, amiloride-sensitive sodium absorption is impaired in inflammatory bowel diseases, in which the response to aldosterone is also blunted. In sharp contrast, amiloride-sensitive sodium absorption is preserved in a non-inflammatory condition such as diverticulosis. Present results suggest that the lack of electrogenic sodium absorption in epithelia from sodium-loaded rats may be entirely attributed to low endogenous serum aldosterone levels, since the response to exogenous aldosterone is preserved.

Interestingly, in the rat distal colon, aldosterone not only stimulates sodium absorption, but simultaneously decreases chloride secretion. Aldosterone activates ATP-dependent potassium channels while inhibiting Ca$^{2+}$-activated potas-
Aldosterone increases rectal O2 consumption

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sium channels, which are, respectively, necessary for electrogenic sodium absorption and electrogenic chloride secretion.24 These contrasting effects on potassium channels have also been demonstrated in the human colon.25,26

The concurrent stimulation of absorption and decrease in secretion makes sense as an energy-efficient mechanism to retain sodium chloride and water. In whole animals, its expected consequence is an increased dehydration of feces. Constipation is one of the symptoms of hyperaldosteronism. It is usually thought to be caused by hypokalemia, because of its inhibitory effect on intestinal transit.27 However, excessive fecal desiccation induced by the direct action of aldosterone on the colonic epithelium might also play a role.

In summary, present results show first, that ENaC blockade proportionally lowers IS, and QO2 in rectal epithelia of both rats submitted to either a normal sodium diet or a low-sodium diet, with a larger effect in the latter. No such effects were noticed in epithelial samples from sodium-loaded rats. Second, aldosterone increases IS, and QO2 of the rat rectal epithelium from animals submitted to standard, low-sodium and high-sodium diets. Third, although aldosterone-induced increases in IS and QO2 were smaller in the sodium-deprived group, which started at higher baseline levels, this group showed the steepest relationship between change in IS and change in QO2, suggesting that chronic sodium deprivation might increase the efficiency of epithelial sodium absorption.

Conflict of interest. None.

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References

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