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Sociedad Argentina de Gastroenterología
Buenos Aires, Argentina

Available in: http://www.redalyc.org/articulo.oa?id=199317328004
Increased oxygen consumption caused by cAMP- and Ca\(^{2+}\)-mediated chloride secretion in rat distal colon

Fernando D. Saravi, Liliana M. Cincunegui, Teobaldo A. Saldeña, Graciela E. Carra, Jorge E. Ibáñez*


Summary
Epithelial ion transport is dependent on ATP supply provided by aerobic metabolism. In the rat distal colon chloride secretion accounts for the largest portion of electrogenic transport measured as the short-circuit current (I\(_{SC}\)). Inhibition of basal chloride secretion decreases epithelial oxygen consumption (QO\(_2\)) in this tissue, while serotonin (5-hydroxytryptamine) proportionally increases both I\(_{sc}\) and QO\(_2\). The effect of serotonin in this tissue is mainly mediated by 5HT4 receptors linked to adenylate cyclase through a stimulant G protein (GS). This work assessed whether the chloride secretion-induced increase in QO\(_2\) is a common characteristic of secretagogues, which act through either cAMP-dependent or Ca\(^{2+}\)-dependent mechanisms. The effects of phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) and muscarinic agonist carbachol (both 0.1 mmol/L) were studied in rat distal colon isolated mucosa mounted in an Ussing chamber adapted for continuous measurement of oxygen consumption, allowing determination of QO\(_2\). Baseline I\(_{SC}\) and QO\(_2\) were compared with I\(_{SC}\) and QO\(_2\) after addition of either serotonin as an active control, IBMX, carbachol or IBMX plus carbachol. Each drug increased proportionally I\(_{sc}\) and QO\(_2\). Although the effect of IBMX alone was modest and that of carbachol was short-lived, a synergic effect on I\(_{sc}\) and QO\(_2\) was seen when both drugs were simultaneously added. Linear regression analysis showed a significant correlation between increases in I\(_{SC}\) and QO\(_2\) (\(r^2 = 0.746; P < 0.0001\)). Thus, stimulation of chloride secretion increases QO\(_2\) regardless of the intracellular pathway involved. These results extend previous findings, corroborating the close coupling between chloride secretion and QO\(_2\) in this epithelium.

Index (palabras claves): chloride secretion, oxygen consumption, descending colon

Resumen
Aumento del consumo de oxígeno causado por la secreción de cloruro mediada por cAMP y Ca\(^{2+}\) en el colon distal de rata

El transporte iónico epitelial exige aporte de ATP provisto por el metabolismo aeróbico. En el colon distal de rata, la secreción de cloruro explica la mayor parte del transporte electrogeno medido como corriente de cortocircuito (I\(_{SC}\)). La inhibición de la secreción basal de cloruro reduce el consumo epitelial de oxígeno (QO\(_2\)), mientras que la serotonina aumenta proporcionalmente I\(_{SC}\) y QO\(_2\). El efecto de la serotonina está mediado por receptores 5HT4 acoplados a adenilato ciclasa mediante proteína G estimulante (GS). En este trabajo se estudió si el aumento del QO\(_2\) asociado con la secreción de cloruro es un efecto común a otros agentes que actúan sobre cAMP o Ca\(^{2+}\). Los efectos del inhibidor de la fosfodiesterasa, 3-isobutil-1-metilxantina (IBMX) y del agonista muscarínico carbachol (ambos a 0.1 mmol/L) se evaluaron en la mucosa aislada del colon distal de rata montado en una cámara de Ussing modificada para determinación continua de la concentración de oxígeno, permitiendo medir QO\(_2\). Se compararon la I\(_{SC}\) y el QO\(_2\) basales con los resultantes del añadido de serotonina (control activo), IBMX, carba-
col, o IBMX y carbacol. Todos aumentaron proporcionalmente $I_{SC}$ y $QO_2$. Aunque el efecto de IBMX solo fue modesto y el del carbacol fue breve, se observó una sinergia cuando fueron agregados simultáneamente. El análisis de regresión lineal mostró una correlación significativa entre los incrementos de $I_{SC}$ y de $QO_2$ ($r^2 = 0.746; P < 0.0001$). Por tanto, la estimulación de la secreción de cloro aumenta el $QO_2$ independientemente de la vía efectora intracelular involucrada. Estos resultados corroboran el estrecho acoplamiento entre secreción de cloro y $QO_2$ en este epitelio.

In most organs with epithelial linings, ion transport is closely coupled to aerobic metabolism. Although several reports on the energetics of transport in mammalian epithelia have been published, there is a paucity of studies assessing the relationship between ion transport and oxygen consumption rate ($QO_2$) in the intestinal mucosa under conditions that preserve vectorial transepithelial ion transport.

Several reports have firmly established that the intestinal epithelium is highly sensitive to hypoxia. Since derangements of epithelial transport are prominent in hypoxic injuries caused by intestinal ischemia or necrotizing enterocolitis and are also an obstacle for the storing of intestinal segments destined to transplantation, the knowledge of the energetic requirements of vectorial transport are pertinent not just to basic science, but probably have also long-ranging clinical implications.

Using the rat distal colon epithelium as a model, we have previously shown a close coupling between $QO_2$ and energetic transport measured as the short-circuit current ($I_{SC}$). In the isolated epithelium mounted in an Ussing chamber, basal chloride secretion demands about 25% of the total epithelial $QO_2$. When chloride secretion is stimulated with serotonin (5-hydroxytryptamine), total $QO_2$ is increased and the fraction of $QO_2$ devoted to chloride secretion reaches about 40% of total consumption. The effect of serotonin on intestinal chloride secretion is due to stimulation of adenylate cyclase. We are not aware of any reports on the effect of other secretagogues on $QO_2$.

This work aimed to determine whether the above mentioned finding may be generalized to other chloride secretagogues acting through different mechanisms. Therefore, in the present report the effect of serotonin on $I_{SC}$ and $QO_2$ was compared with those of a secretagogue that raises intracellular Ca$^{2+}$ and a secretagogue that reduces cAMP degradation, namely the cholinergic muscarinic agonist carbacol, and the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX), respectively. Additionally, the interaction between these drugs was assessed.

**Material and methods**

**Animals.** Wistar-Hokkaido male rats (250-300 g body weight) were housed and managed according to the guidelines for animal care and biosafety of our Medical School. The animals were kept at an environmental temperature of 25 ± 1°C with a 12-hr light-dark cycle, and fed with standard rat chow (Carhill Co.) and tap water ad libitum.

**Dissection and Mounting.** Rats were anesthetized with diethyl ether. The entire colon was removed and rinsed free of contents with cold Ringer solution. An 1-cm segment was cut from the descending portion just above the pelvic brim and dissected as previously described to obtain an isolated mucosa preparation. Then, the segment was cut open along its mesenteric border and extended as a flat sheet. The adherent mucus gel layer was gently removed with a cotton tip soaked in Ringer solution. The tissue was mounted in a Ussing-type chamber, which is described below.

**Solution and Gas Mixtures.** The Ringer solution had the following composition (in mmol/L): Na, 132.8; K, 4.5; Ca, 1.25; Mg, 1; Cl, 114; HCO$_3$, 24; H$_3$PO$_4$, 1; SO$_4$, 1; D (+) glucose, 10. Solution osmolality was 280 mOsm/kg H$_2$O and its pH was 7.40 when gassed with 95% O$_2$ and 5% CO$_2$. The gas mixture was purchased from Air Liquide, Inc. and had its composition certified by the company.

**Drugs.** To prevent bacterial overgrowth during the course of experiments, gentamicin (Schering-Plough) was added to both hemichambers at the start of each experiment for a final concentration of 91 mg/mL. Carbachol, IBMX and serotonin were purchased from Sigma. Drug solutions were freshly prepared for each experiment. IBMX was dissolved in dimethylsulfoxide, while serotonin and carbachol were dissolved in Ringer solution. Drugs were added as indicated to the serosal hemichamber to achieve a concentration of 0.1 mmol/L.

**Modified Ussing Chamber and Electrical Measurements.** The modified Ussing chamber employed in this work (Figure 1) has been previously validated. Briefly, the chamber has an opening of 1
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...was measured for 30-min periods under baseline conditions.

Tissues were divided into four groups (each with \(n = 6\)) and treated with serotonin (active control), IBMX, carbachol or IBMX plus carbachol. In all cases treatment \(\text{QO}_2\) was measured for a 30-min period starting immediately after adding the drug. \(\text{QO}_2\) was calculated from the sum of changes in oxygen concentration in each hemichamber during the measurement period, as previously described.\(^4\) Reported \(I_{SC}\) is the average recorded during the corresponding \(\text{QO}_2\) measurement period.

After each experiment, a blank run was performed after removing the mucosal sheet and replacing it with a polyethylene membrane. The experiment was discarded if the decrease in oxygen concentration was above 10% of that observed with the biological sample.

**Statistics.** Two-sided, paired Student’s \(t\) tests were performed to assess the effect of each drug on \(I_{SC}\) and \(\text{QO}_2\). A one-way analysis of variance followed by Dunnett’s multiple comparison test was used to compare the effects of carbachol, IBMX, and carbachol plus IBMX with those of serotonin. The relationship between the change in \(I_{SC}\) and the change in \(\text{QO}_2\) was assessed with linear regression, checking for significant deviations from linearity. All analyses were performed with a standard commercial software (GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego, California, USA). Unless otherwise stated, values are reported as mean ± sem. Values of \(P\) lower than 0.05 were considered as significant.

**Results**

As previously reported, tissues mounted in the modified Using chamber had a very good stability throughout the experimental period. Baseline values for all mucosal samples (\(n = 24\)) were: \(I_{SC} = 71.1 ± 2.5\) mA cm\(^{-2}\) (2.65 ± 0.09 mmol.h\(^{-1}\).cm\(^{-2}\)) transepithelial potential difference = 6.48 ± 0.30 mV; transepithelial resistivity = 90.7 ± 2.2 W.cm\(^{-2}\) and \(\text{QO}_2 = 2.78 ± 0.04\) mmol.h\(^{-1}\).cm\(^{-2}\).

Typical \(I_{SC}\) responses of the isolated mucosa to serotonin, carbachol, IBMX and carbachol plus IBMX are shown in Figure 2. Serotonin evoked the largest \(I_{SC}\) increase. Carbachol caused a sharp but...
short-lived peak of $I_{SC}$, while the effect of IBMX was slower but longer-lasting. Finally, simultaneous administration of carbachol and IBMX evoked a

rise in $I_{SC}$ with a peak followed by a high, lasting plateau.

$I_{SC}$ and $QO_2$ before secretagogue addition and after it are presented in Table 1. Both IBMX and carbachol augmented $I_{SC}$ and $QO_2$. The response to IBMX was smaller than that observed with serotonin. On the other hand, the response to carbachol was short-lived when compared with the effect of serotonin. However, when IBMX and carbachol were simultaneously added, a synergistic effect was achieved regarding both magnitude and duration of the increases in $I_{SC}$ and $QO_2$. As shown in Figure 3, the combined effects of carbachol plus IBMX were not significantly different from those of serotonin.

**Table 1.** Changes in short-circuit current (ISC) and oxygen consumption rate (QO2) caused by chloride secretagogues in rat distal colon isolated mucosa. IBMX, 3-isobutyl-1-methylxanthine. Figures are mean ± sem.

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>ISC (mol.cm⁻².h⁻¹)</th>
<th>QO2 (mol.cm⁻².h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>Treatment</td>
</tr>
<tr>
<td>Serotonin</td>
<td>6</td>
<td>2.71 ± 0.19</td>
<td>4.17 ± 0.27*</td>
</tr>
<tr>
<td>IBMX</td>
<td>6</td>
<td>2.54 ± 0.21</td>
<td>2.89 ± 0.23§</td>
</tr>
<tr>
<td>Carbachol</td>
<td>6</td>
<td>2.69 ± 0.22</td>
<td>3.39 ± 0.25*</td>
</tr>
<tr>
<td>Carbachol + IBMX</td>
<td>6</td>
<td>2.67 ± 0.15</td>
<td>4.05 ± 0.43*</td>
</tr>
</tbody>
</table>

Compared with the respective baseline: *P < 0.0001; §P = 0.0016; #P = 0.0003; &P = 0.0011.

**Figure 2.** Typical short-circuit current (ISC) responses of rat distal colon isolated mucosa to chloride secretagogues from actual representative experiments. The arrows indicate addition of the indicated drug. IBMX, 3-isobutyl-1-methylxanthine.

**Figure 3.** Mean changes and sem (n = 6 for each treatment) in short-circuit current (D ISC, upper panel) and oxygen consumption rate (D QO2, lower panel). IBMX, 3-isobutyl-1-methylxanthine; carb, carbachol. Data analyzed by one-way ANOVA followed by Dunnett’s multiple comparisons test: *P < 0.0001 compared with serotonin; §P > 0.05 compared with serotonin.
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Figure 4. Results of linear regression analysis of changes in short-circuit current (D ISC) and oxygen consumption rate (D QO2) in response to chloride secretagogues. IBMX, 3-isobutyl-1-methylxanthine; carb, carbachol. Dashed lines enclose the 95% confidence limits. The slope is 0.1906 ± 0.02371; r² = 0.7461; P < 0.0001.

increase in QO₂ with r = 0.864 and a slope of 0.1906 ± 0.0237 (Figure 4).

Discussion

Secretion of chloride is the main process accounting for the ISC of the unstimulated rat distal colon epithelium in vitro. Chloride secretion can be increased with many chemicals including neurotransmitters, hormones, autacoids, toxins, drugs and natural products, as recently reviewed. Two prominent classes of secretagogues are serotonergic agonists and muscarinic agonists.

In the distal colon, serotonin increases chloride secretion mainly through activation of 5-HT4 receptors although 5-HT3 receptors also play a role. These receptors are coupled to adenylate cyclase through a stimulating G protein (Gs) and therefore their activation leads to increased cAMP production and intracellular concentration. IBMX is also able to raise intracellular cAMP concentration but through a different mechanism, namely reducing its degradation through inhibition of the nucleotide-degrading enzyme phosphodiesterase. In either case, activation of cAMP-dependent kinase then leads to phosphorylation of apical cystic fibrosis transmembrane conductance regulator (CFTR) chloride channels and opening of basolateral K⁺ channels known as KvLQT1, resulting in increased chloride secretion.

Muscarinic agonists like carbachol act at receptors coupled by G proteins (Gq) to phospholipase C, resulting in increased production of inositol triphosphate and diacylglycerol. Inositol triphosphate stimulates Ca²⁺ release, which in turn opens basolateral Ca²⁺-sensitive K⁺ channels, causing membrane depolarization. This depolarization alters the electrochemical equilibrium potential for Cl⁻ leading to chloride secretion. Therefore, chloride secretion stimulation by carbachol is largely indirect, through their effect on membrane potential, although an effect on the recently described Ca²⁺-sensitive apical Cl⁻ channels is likely. At any rate, the carbachol seems to need some background activation of apical chloride channels by cAMP in order to cause increased chloride secretion. Consequently, this effect of carbachol is potentiated by agents that raise intracellular cAMP concentration.

Besides open apical Cl⁻ channels, epithelial chloride secretion demands a mechanism allowing basolateral entry of chloride ions. The main transporter involved is the loop diuretic-sensitive Na,K,2Cl sympporter. This is a secondary active transport powered by the Na⁺ electrochemical gradient, which in turn is maintained by the Na,K-ATPase (sodium pump) located at the basolateral membrane. When the Na,K-ATPase is inhibited by ouabain or shortage of ATP supply as in hypoxia, chloride secretion decreases. Conversely, stimulants of chloride secretion leading to higher basolateral Na, K, 2 Cl-sympporter activity may be expected to require an increased Na,K-ATPase turnover rate, a larger ATP supply and therefore higher QO₂.

The present results demonstrate that all three chloride secretagogues tested, which work through different intracellular effector mechanisms, concomitantly raise both ISC and QO₂. Furthermore, the effect on QO₂ is proportional to the magnitude of the increase in chloride secretion, suggesting a close coupling of chloride secretion and oxygen consumption that may help to explain the derangement of ion transport seen in intestinal disorders like, for example, inflammatory bowel disease.

References
2. Silverthorn SU, Eaton DC. Respiration and sodium trans-