Dig Dis Sci (2013) 58:2499–2506
DOI 10.1007/s10620-013-2711-0

ORIGINAL ARTICLE

The Effect of Acute Hypoxia on Short-Circuit Current and Epithelial Resistivity in Biopsies from Human Colon

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Received: 15 December 2012 / Accepted: 2 May 2013 / Published online: 22 May 2013

Abstract
Background and Aims In isolated colonic mucosa, decreases in short-circuit current (I_{SC}) and transepithelial resistivity (R_{TE}) occur when hypoxia is either induced at both sides or only at the serosal side of the epithelium. We assessed in human colon biopsies the sensitivity to serosal-only hypoxia and mucosal-only hypoxia and whether Na, K-ATPase blockade with ouabain interacts with hypoxia.
Materials and Methods Biopsy material from patients undergoing colonoscopy was mounted in an Ussing chamber for small samples (1-mm^2 window). In a series of experiments we assessed viability and the electrical response to the mucolytic, dithiothreitol (1 mmol/l). In a second series, we explored the effect of hypoxia without and with ouabain. In a third series, we evaluated the response to a cycle of hypoxia and reoxygenation induced at the serosal or mucosal side while keeping the oxygenation of the opposite side.
Results 1st series: Dithiothreitol significantly decreased the unstirred layer and I_{SC} but increased R_{TE}. 2nd series: Both hypoxia and ouabain decreased I_{SC}, but ouabain increased R_{TE} and this effect on R_{TE} prevailed even during hypoxia. 3rd series: Mucosal hypoxia caused lesser decreases of I_{SC} and R_{TE} than serosal hypoxia; in the former, but not in the latter, recovery was complete upon reoxygenation.
Conclusions In mucolytic concentration, dithiothreitol modifies I_{SC} and R_{TE}. Oxygen supply from the serosal side is more important to sustain I_{SC} and R_{TE} in biopsy samples. The different effect of hypoxia and Na, K-ATPase blockade on R_{TE} suggests that their depressing effect on I_{SC} involves different mechanisms.

Keywords Dithiothreitol · Human colon · Hypoxia · Ouabain · Unstirred layer · Ussing chamber

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Introduction

The conservation of water and electrolytes is a major function of the colon [1, 2]. Absorption of most of the water, sodium, and bicarbonate that crosses the ileocecal valve is efficiently accomplished by the colonic epithelium.

As in other tissues, the absorption of water in the colon is made possible by osmotic gradients generated by net transepithelial solute transport. Epithelial ion transport depends on oxidative phosphorylation [3] and therefore demands an adequate oxygen supply, since colonocyte energy metabolism is largely aerobic, regardless of which precise substrate is actually employed as a metabolic fuel [4–6]. As a consequence, ion transport is quite sensitive to hypoxia [7–10].

Hypoxia is the earliest injuring factor during intestinal ischemia, with a major effect on the mucosa [11, 12]. Experimentally, hypoxia induces pathological changes resembling those caused by necrotizing enterocolitis [13] and in rat pups these changes may be observed after a single 5-min episode of severe hypoxia [14]. Clinically, hypoxia may be a cause of intestinal dysfunction during anesthesia, prompting the development of different methods for the in vivo assessment of mucosal oxygenation [15].

The distal colon lumen is an essentially anaerobic medium with very low oxygen pressure [16, 17]. Therefore, most of the oxygen supply in vivo must come from the submucosal blood vessels, reaching the epithelium from its serosal side [18, 19]. A different situation is found in vitro, when the epithelium is dissected, isolated, and mounted as a flat sheet in an Ussing chamber. This arrangement allows oxygen to be supplied at the same partial pressure from both the mucosal and the serosal sides. However, even in this situation, oxygen supply from the serosal side seems more important than that from the mucosal side to sustain electrogenic ion transport both in rat [9] and human colon [10]. This is shown by the fact that hypoxia induced at the serosal side of the epithelium, while keeping constant the oxygenation at the mucosal side, causes a reduction of short-circuit current similar to that caused by hypoxia induced at both sides simultaneously; this depressing effect is not seen when hypoxia is induced at the mucosal side while keeping constant the oxygenation from the serosal side. Therefore, in the isolated mucosa, serosal oxygen supply is both necessary and sufficient to sustain electrogenic ion transport, which is not the case for oxygen supply from the mucosal side.

In vitro, several barriers might hinder oxygen diffusion from the bulk solution to the epithelial cells. One of these is the adherent mucus gel layer [20]. Dithiothreitol is the agent most commonly used, in concentrations ranging from 1 to 10 mmol/l, to dissolve the mucus gel since its introduction for this purpose almost 40 years ago [21]. However, previous work has shown that dithiothreitol modifies I_{SC} and transepithelial resistivity (R_{TE}) in rat distal colon [22, 23].

In the present study, we assessed the effect of dithiothreitol on electrophysiological variables, the interaction of the effects of hypoxia and Na, K-ATPase blockade, and whether the response to cycles of serosal or mucosal hypoxia and reoxygenation are also observed in samples from human colon biopsies.

Subjects and Methods

Subjects

With informed consent, biopsy material was obtained from patients with average risk for colorectal cancer, undergoing elective diagnostic colonoscopy. Individuals with a history of inflammatory bowel disease, or anticoagulated at the time of the study were excluded. The study was performed in full compliance with the last edition (2008) of the Declaration of Helsinki. The protocol was evaluated and approved by the Committee on Bioethics of the Faculty of Medical Sciences.

Biopsy samples from 30 patients (14 women) were studied. Thirteen patients (seven female) had arterial hypertension, treated with inhibitors of the angiotensin converting enzyme or antagonists of the angiotensin II AT1 receptor (plus a thiazide diuretic in four cases). Nine patients (all female) had moderate osteoarthritis and were regularly taking non-steroid antiinflammatory drugs. Three patients (one female) had type 2 diabetes, medicated with metformin alone or metformin plus sitagliptin. Three female patients had suffered from breast cancer 10–15 years before this study, treated at that time, with no current sign of recurrence. Three females were taking selective inhibitors of serotonin uptake. There were no complications related to the colonoscopic procedure.

Tissue Preparation and Mounting

In each patient, two biopsies for experimental purposes were obtained from macroscopically healthy sigmoid colon mucosa at a length 25–35 cm orally from the anus with reusable round cup biopsy forceps Olympus FB-25 K-1. Samples had an epithelial surface of approximately 2 mm^2. They were rinsed and placed in a sterile reservoir containing a transport solution (see below) saturated with oxygen and kept at 4 °C, until their arrival to the laboratory. At the laboratory, samples were rinsed again, this time with the same solution used during the experiment (see below), and carefully placed in an Ussing chamber for small samples, with a 1-mm^2 window, built in our workshop according to directions provided by Dr. Marcus Mall (pers. comm.). The device for holding the samples is shown in Fig. 1. Its thickness around the window is 1 mm. It was modeled in acrylic by one of the authors (JEI) with a lathe, using a special thin drill for the window. The surface immediately around the window was deliberately left incompletely polished, as we found that a slightly rough surface allowed adequate tissue sample adherence to the support without any adhesive. During the experiments, the chamber was kept at 37 °C with a water circulating system carved within its wall thickness. The chamber content was continually gassed with oxygen except for brief periods of hypoxia. I_{SC} and transepithelial potential difference (V_{TE}) were measured and R_{TE} was calculated as previously described [24].

Histological Examination

After each experiment, the biopsy sample was fixed in 4 % buffered paraformaldehyde, and prepared for light microscopy with hematoxylin and eosin (H&E) staining, and photographed with a Nikon DF-I1 camera coupled to a Nikon 80-I microscope (Nikon Instruments, Tokyo, Japan). For comparison purposes, some intact samples that were

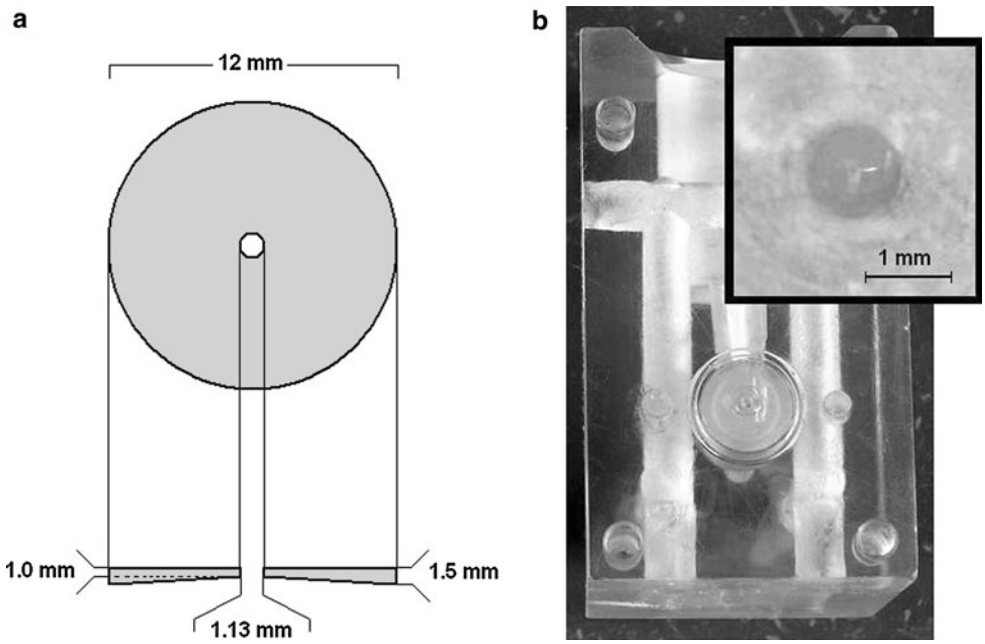

Fig. 1 Ussing chamber for small samples **a** scheme of the support for holding biopsies, **b** front view of one hemichamber showing the support in position; the *insert* shows an amplification of the center of the support

not mounted in the Ussing chamber were similarly examined. We assessed the presence of pathological changes, the thickness of the sample, and the extent of edge damage.

Solutions, Gases, and Drugs

The transport solution had the following composition: 127 mM NaCl, 5 mM KCl, 1.00 mM $MgCl_2$, 1.25 mM $CaCl_2$, 10 mM HEPES, 5 mM sodium pyruvate, 5 mM D-glucose and 10 g/l bovine albumin. The Ringer solution employed during dissection and for Ussing chamber experiments contained 145 mM NaCl, 1.6 mM K_2HPO_4, 0.6 KH_2PO_4, 1.00 $MgCl_2$, 1.30 $CaCl_2$, 5 mM D-glucose, and 91 μg/ml gentamicin (to prevent bacterial overgrowth). Dithiothreitol, amiloride, and ouabain were purchased from Sigma-Aldrich and gentamicin from Schering-Plough. Oxygen and nitrogen were provided by AirLiquide Argentina, Inc.

Experimental Procedures

In the first series of experiments, the effect of dithiothreitol was assessed by adding it to the mucosal hemichamber for a final concentration of 1 mmol/l and comparing I_{SC}, V_{TE}, and R_{TE} immediately before and 10 min after the addition of the mucolytic agent. The effect of dithiothreitol as a mucolytic was assessed by calculation of the unstirred layer according to Diamond [25], measuring the half time for the effect of the epithelial sodium channel blocker, amiloride (0.1 mmol/L), added at the mucosal hemichamber. We used the value of 7. 10^{-6} cm^2/s as the diffusion coefficient of amiloride [26]. We did not take into account the reaction time of the drug with epithelial sodium channels, since it may be deemed negligible compared with diffusion time [27]. The thickness of the unstirred layer was therefore calculated as the square root of D. T½/0.38, where D is the diffusion coefficient of amiloride and T½ the half time. In the second series of experiments, hypoxia was induced by switching the gas from oxygen to nitrogen in both sides of the chamber for 2 min, before or after the addition of ouabain (1 mmol/l) to the serosal side. Reoxygenation was accomplished by switching back to oxygen. In the third series, unilateral hypoxia was induced by switching the gas from oxygen to nitrogen in one side of the chamber (in random order) for 2 min, while maintaining the oxygenation at the opposite side. The oxygen supply to the hypoxic hemichamber was restored on reoxygenation.

Statistical Analysis

The effect of dithiothreitol was evaluated with a paired, two-sided, Student's *t* test. The effect of hypoxia and reoxygenation, either with the addition of ouabain or without it, was assessed with ANOVA followed by Tukey's test to compare the means for each group, or Dunnett's test to compare the basal values with the values attained after each treatment. Before Student's *t* test and ANOVA, a Kolmogorov–Smirnov's test was applied to make sure that the distribution did not significantly depart from a Gaussian one.

Results were analyzed with the commercial statistical software Prism 5.4 for Windows (GraphPad Inc., San Diego, CA, USA). Values are expressed as mean ± SEM. In post hoc analyses, the 95 % confidence interval (CI_{95}) is

presented for each significant difference. Values of $p < 0.05$ were considered significant.

Results

Histological examination of the mounted samples showed in most cases preservation of structure with little edge damage (Fig. 2). All samples included the full thickness epithelium, the laminae propria, the muscularis mucosae and remains of submucosa. The results of four experiments were not taken into account because of the presence of an inflammatory infiltrate ($n = 2$) or extensive edge damage ($n = 2$).

After the sample was mounted in the Ussing chamber, I_{SC} reached a stable value 30–60 min, which was about the same time it took for the content of the chamber to reach the 37 °C working temperature. Dithiothreitol did remove the adherent mucus gel layer. The half time for the effect of amiloride on I_{SC} was 54.3 ± 4.5 s under control conditions and decreased to 22.7 ± 3.9 s in the presence of dithiothreitol (Fig. 3). The calculated unstirred layer was 316 μm for the control samples and 204 μm for those pre-treated with dithiothreitol. As shown in Table 1, dithiothreitol also caused a reduction of I_{SC} and V_{TE} but increased R_{TE}. These effects persisted even after removing the content of the hemichamber and filling it with dithiothreitol-free solution (data not shown). Given these results, we did not use dithiothreitol as a mucolytic in the other experimental series.

The effect of adding ouabain after a cycle of hypoxia and reoxygenation is presented in Table 2. A 2-min hypoxia induced at both sides of the chamber depressed I_{SC}, V_{TE}, and R_{TE}, with complete recovery on reoxygenation. Subsequent addition of ouabain at the serosal side of the chamber caused a reduction of I_{SC} and V_{TE} but was associated with an increase in R_{TE}. The latter persisted even after a new cycle of hypoxia and reoxygenation. If ouabain was added before inducing hypoxia, then both I_{SC} and V_{TE} decreased while R_{TE} increased, and a cycle of hypoxia and reoxygenation did not cause significant further changes in any variable (Table 3).

The effects of inducing a 2-min unilateral hypoxia at either the mucosal or the serosal hemichamber, followed by reoxygenation of the hypoxic side, are presented in Table 4. The values for hypoxia and reoxygenation are, respectively, the lowest and highest reached during each maneuver. Hypoxia at either side caused a decrease of I_{SC} but the decrease, assessed by a two-sided Student's t test, was smaller during mucosal hypoxia than during serosal hypoxia ($p = 0.0191$). Likewise, hypoxia at either side depressed V_{TE} and R_{TE}, but the decreases were smaller after mucosal than after serosal hypoxia; respectively $p = 0.0282$ and $p = 0.004$ for the differences. Furthermore, after mucosal hypoxia I_{SC}, V_{TE}, and R_{TE} showed complete recovery upon reoxygenation, but after serosal hypoxia only I_{SC} showed complete recovery, while V_{TE} and R_{TE} remained below the basal value.

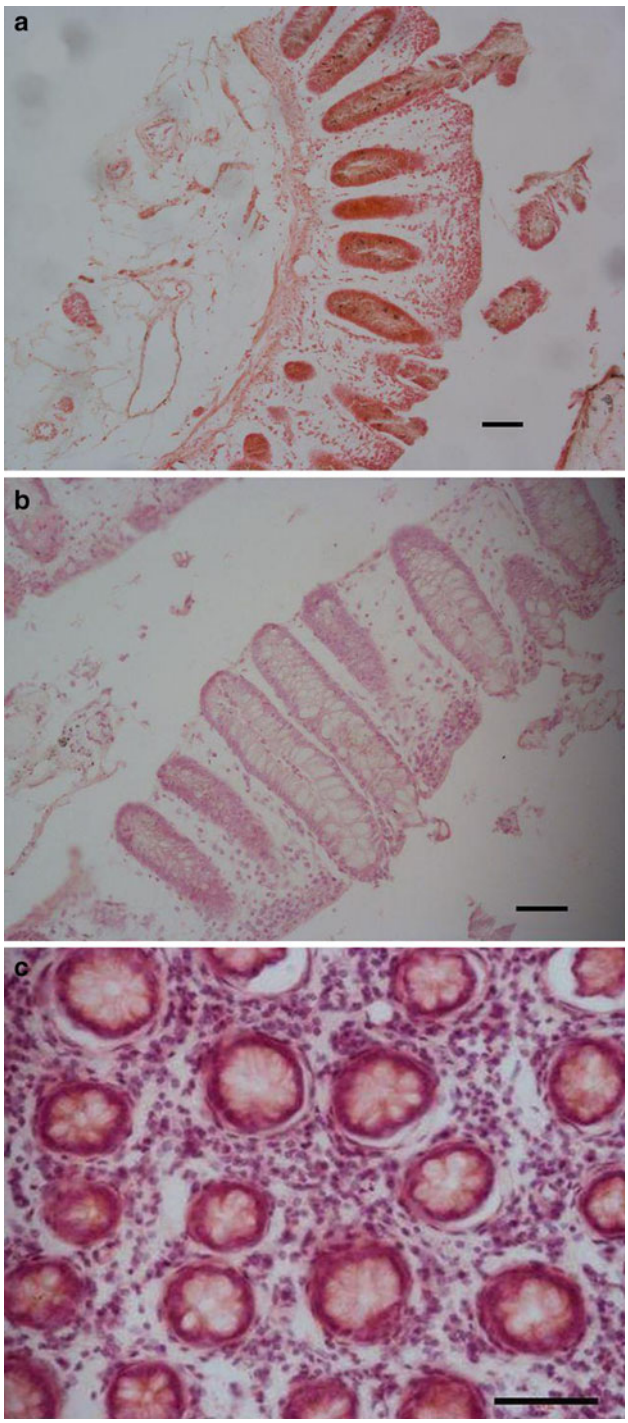

Fig. 2 Light microscopy of biopsy samples **a** biopsy fixed without being mounted in the Ussing chamber, **b** and **c** biopsies fixed after 2 h in the Ussing chamber. Hematoxylin and eosin (H&E). *Calibration bars* = 50 μm

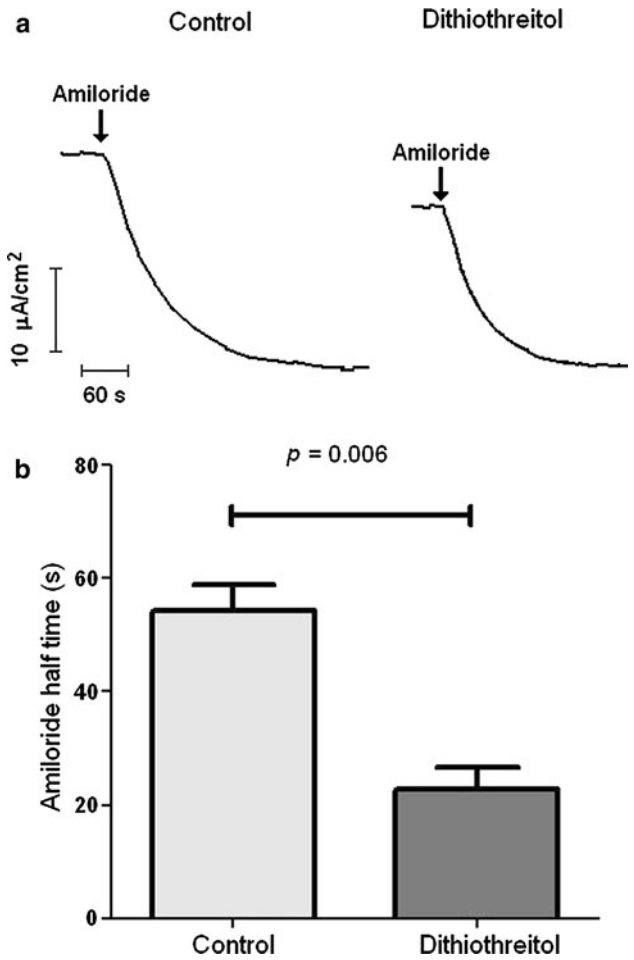

Fig. 3 Assessment of the half time of the depressing effect of amiloride (0.1 mmol/l) on I_{SC} under control condition and in the presence of dithiothreitol (1 mmol/l) **a** representative I_{SC} recordings, **b** results were analyzed with an unpaired, two-sided, Student's *t* test

Table 1 Basal values and effect of dithiothreitol in human colon biopsies

	I_{SC} (μA/cm^2)	V_{TE} (mV)	R_{TE} (Ω cm^2)
Basal	44.9 ± 2.6	1.14 ± 0.08	25.5 ± 1.5
Dithiothreitol	25.7 ± 1.5	0.72 ± 0.06	28.1 ± 1.5
p	0.0002	0.0003	0.0223

Dithiothreitol was added to the mucosal hemichamber at a final concentration of 1 mmol/l. Results were analyzed with a paired, two-sided, Student's *t* test ($n = 6$)

Discussion

The basal values for I_{SC} and R_{TE} recorded in our experiments are well within the range found by other groups working with human colon biopsies. For example, in rectal biopsies, Mall et al. reported mean values of 52.3 μA cm^{-2} and 27.8 Ω cm^2 [28], 33.4 μA cm^{-2} and 27.8 Ω cm^2 [29], and 58.2 μA cm^{-2} and 23.7 Ω cm^2 [30], while Park et al. reported mean values of 51.4 μA cm^{-2} and 20.4 Ω cm^2 [31]. Other authors found similar values of I_{SC} in the human sigmoid colon, but lower values of R_{TE}; for example, 54.7 μA cm^{-2} and 17.0 Ω cm^2 [32], 43.8 μA cm^{-2} and 13.0 Ω cm^2 [33], and 38.1 μA cm^{-2} and 11.0 Ω cm^2 [34]. These data, plus the histological confirmation of the integrity of the samples after each experiment, corroborate the validity of our results.

In human colon biopsies, we only assessed the effect of dithiothreitol at its minimal mucolytic concentration (1 mmol/l). We found that it was indeed effective as a mucolytic, as indicated by a 112 μm decrease in the calculated unstirred layer, a result which is in good agreement with the 134-μm thickness of the adherent mucus gel layer of the human left colon reported by Pullan et al. [35]. However, dithiothreitol also reduced I_{SC} and actually increased R_{TE}, as do lower concentrations in the rat distal colon. Therefore, we avoided using dithiothreitol in the

Table 2 The effect of hypoxia-reoxygenation followed by ouabain and a new cycle of hypoxia-reoxygenation of electrophysiological variables

Variable	Condition		
	I_{SC} (μA/cm^2)[a]	V_{TE} (mV)[b]	R_{TE} (Ω cm^2)[c]
Basal	34.2 ± 4.2	2.87 ± 0.26	85.3 ± 4.9
Hypoxia	24.0 ± 3.6	1.61 ± 0.19	66.7 ± 6,5
Reoxygenation	34.4 ± 4.3	2.87 ± 0.24	86.5 ± 3.8
Ouabain	12.7 ± 1.1	1.24 ± 0.07	97.8 ± 4.3
Post-ouabain hypoxia	9.0 ± 0.9	0.88 ± 0.03	98.2 ± 2.8
Post-ouabain reoxygenation	10.0 ± 0.8	0.93 ± 0.03	93.3 ± 4.0

Differences were assessed by repeated measures ANOVA followed by Tukey's test ($n = 5$)

[a] $p < 0.0001$. Differences between groups were significant ($p < 0.05$) with the following exceptions: basal vs. reoxygenation (CI_{95} −9.178 to 8.822); ouabain versus post-ouabain hypoxia (CI_{95} −5.280 to 12.72); ouabain versus post-ouabain reoxygenation (CI_{95} −6.240 to 11.76) and post-ouabain hypoxia versus post-ouabain reoxygenation (CI_{95} −9.960 to 8.040)

[b] $p < 0.0001$. Differences between groups were significant ($p < 0.05$) with the following exceptions: basal versus reoxygenation (CI_{95} −0.5478 to 0.5318); hypoxia versus ouabain (CI_{95} −0.1738 to 0.9058); ouabain versus post-ouabain hypoxia (CI_{95} −0.1778 to 0.9018); ouabain versus post-ouabain reoxygenation (CI_{95} −0.2558 to 0.8538) and post-ouabain hypoxia versus post-ouabain reoxygenation (CI_{95} −0.5878 to 0.4918)

[c] $p < 0.0001$. Differences between groups were significant ($p < 0.05$) with the following exceptions: basal versus reoxygenation (CI_{95} −12.01 to 9.728); basal versus post-ouabain reoxygenation (CI_{95} −18.83 to 2.908); reoxygenation versus post-ouabain reoxygenation (CI_{95} −17.69 to 4.048); ouabain versus post-ouabain hypoxia (CI_{95} −11.05 to 10.69); ouabain versus post-ouabain reoxygenation (CI_{95} −6.348 to 15.39) and post-ouabain hypoxia versus post-ouabain reoxygenation (CI_{95} −6.168 to 15.57)

Table 3 The effect of ouabain applied before inducing hypoxia and reoxygenation

Variables	Condition		
	I_{SC} ($\mu A/cm^2$)[a]	V_{TE} (mV)[b]	R_{TE} ($\Omega\ cm^2$)[c]
Basal	47.1 ± 3.7	3.66 ± 0.36	78.0 ± 5.4
Ouabain	23.0 ± 2.5	2.11 ± 0.26	91.8 ± 7.7
Post-ouabain hypoxia	18.5 ± 1.5	1.62 ± 0.18	87.4 ± 6.5
Post-ouabain reoxygenation	19.5 ± 1.3	1.69 ± 0.19	86.5 ± 6.8

Differences were assessed by repeated measures ANOVA followed by Tukey's test ($n = 5$)

[a] $p < 0.0001$. Differences between groups were significant ($p < 0.05$) with the following exceptions: ouabain versus post-ouabain hypoxia (CI_{95} −0.4375 to 9.517); ouabain versus post-ouabain reoxygenation (CI_{95} −1.477 to 8.477); and post-ouabain hypoxia versus post-ouabain reoxygenation (CI_{95} −6.017 to 3.397)

[b] $p < 0.0001$. Differences between groups were significant ($p < 0.05$) with the following exception: post-ouabain hypoxia versus post-ouabain reoxygenation (CI_{95} −0.4631 to 0.3191)

[c] $p < 0.0001$. Differences between groups were significant ($p < 0.05$) with the following exceptions: ouabain versus post-ouabain hypoxia (CI_{95} −2.407 to 10.43); ouabain versus post-ouabain reoxygenation (CI_{95} −1.039 to 11.79) and post-ouabain hypoxia versus post-ouabain reoxygenation (CI_{95} −5.049 to 7.785)

other experimental series. The persistence of an apparent 204 μm unstirred layer in the presence of dithiothreitol may be due, at least in part, to a limitation of diffusion posed by the 1-mm thickness of the supporting device at the window level.

Since the adherent mucus gel layer poses a significant hindrance for oxygen diffusion [20] and is difficult to remove by mechanical means in biopsies, we assessed whether it could be removed by dithiothreitol without affecting I_{SC} and R_{TE}. Dithiothreitol is a sulfhydryl reactive agent and sulfhydryl and disulfide groups are ubiquitous; therefore, it might be expected to have other effects besides its intended mucolytic action [36–38]. For example, in the gastric mucosa the mucolytic effect of dithiothreitol takes about 1 h to be noticeable, but it collapses the pH gradient within 10 min (Prof. Adrian Allen, pers. comm.).

A study in rat distal colon addressed the concentration dependence of dithiothreitol effect, applied at both the serosal or luminal side of the isolated mucosa mounted in a conventional Ussing chamber [23]. In a concentration range of 1 μmol/l–1 mmol/l, dithiothreitol caused a progressive decrease of I_{SC} to about half of the control value at the highest concentration when applied at the luminal side. On the other hand, the R_{TE} showed a biphasic response. R_{TE} increased progressively within the concentration range of 1 μmol/l–0.5 mmol/l, but dropped to less than 60 % of the control value at 1 mmol/l.

In experiments where hypoxia was induced simultaneously at both sides of the biopsy, the electrophysiological response was similar to that observed in isolated mucosa obtained from surgical pieces [10]: decreases in I_{SC}, V_{TE}, and R_{TE}, which showed complete recovery when the oxygen supply was restored. The addition of ouabain also depressed, as expected, I_{SC} and V_{TE} but caused an increase in R_{TE}, which persisted without significant change even after a new hypoxic challenge. In this work, we have not addressed the mechanism of the R_{TE} increase caused by ouabain, and we are not aware of any study on this topic in human colon. One obvious reason for the R_{TE} increase might be the suppression of the Na, K-ATPase as a pathway for net transepithelial current. However, in the goldfish intestine, Groot et al. [39] concluded that the patency of the space between epithelial cells is maintained by a ouabain-sensitive transport mechanism (presumably the

Table 4 Effect of unilateral hypoxia followed by reoxygenation at either the mucosal or the serosal side of the chamber

Variable	Mucosal-only hypoxia			Serosal-only hypoxia		
	I_{SC} ($\mu A/cm^2$)[a]	V_{TE} (mV)[b]	R_{TE} ($\Omega\ cm^2$)[c]	I_{SC} ($\mu A/cm^2$)[d]	V_{TE} (mV)[e]	R_{TE} ($\Omega\ cm^2$)[f]
Condition						
Basal	32.0 ± 3.0	2.90 ± 0.41	91.0 ± 4.2	32.0 ± 3.0	2.80 ± 0.22	88.0 ± 4.3
Hypoxia	25.0 ± 2.2	2.00 ± 0.22	80.0 ± 4.5	19.0 ± 1.7	1.28 ± 0.41	67.0 ± 2.3
Reoxygenation	30.0 ± 3.1	2.72 ± 0.42	93.0 ± 4.3	29.0 ± 3.3	2.10 ± 0.31	72.4 ± 4.2

Differences were assessed by repeated measures ANOVA followed by Dunnett's ($n = 5$ each group)

[a] $p = 0.0280$; basal versus hypoxia, $CI_{95} = 1.3$ to 12.7; basal versus reoxygenation, $CI_{95} = -3.7$ to 7.7

[b] $p = 0.0068$; basal versus hypoxia, $CI_{95} = 0.32$ to 1.40; basal versus reoxygenation, $CI_{95} = -0.36$ to 0.72

[c] $p = 0.0028$; basal versus hypoxia, $CI_{95} = 3.8$–18.2; basal versus reoxygenation, $CI_{95} = -9.2$ to 5.2

[d] $p = 0.0211$; basal versus hypoxia, $CI_{95} = 2.9$–23.1; basal versus reoxygenation, $CI_{95} = -7.1$–13.1

[e] $p = 0.0008$; basal versus hypoxia, $CI_{95} = 0.89$–2.22; basal versus reoxygenation, $CI_{95} = 0.07$–1.40

[f] $p = 0.0024$; basal versus hypoxia, $CI_{95} = 10.0$–32.0; basal versus reoxygenation, $CI_{95} = 4.6$–26.6

Na, K-ATPase), and the collapse of this space is the main reason for the observed increase in R_{TE} caused by ouabain. In the frog kidney, it was suggested that the reason for changes in R_{TE} caused by ouabain is an indirect effect caused by changes in potassium conductance secondary to Na, K-ATPase inhibition [40]. More recently, it has been reported that in Madin-Darby canine kidney cells (MDCK), ouabain modulates tight junction permeability and causes increases in R_{TE} even in very low concentrations that do not affect the Na, K-ATPase [41]. Given the variety of possible mechanisms, the elucidation of the exact mechanism for the increase in R_{TE} by ouabain in human colon deserves further research. At any rate, once ouabain was present, hypoxia no longer caused any significant reduction of R_{TE}.

An asymmetrical response to unilateral hypoxia induced from either the serosal or the mucosal side of the epithelium was initially reported in rat distal colon isolated mucosa [42] but is also observed in human colon isolated mucosa [10]. While serosal hypoxia caused a depressor effect similar to hypoxia induced at both sides, mucosal hypoxia did not cause any significant effect, provided that oxygenation from the serosal side was maintained. Biopsies showed the same response to serosal hypoxia, but a different response to mucosal hypoxia, which caused a smaller but significant decrease in I_{SC}, V_{TE} and R_{TE}. This may be due to the fact that biopsy material includes submucosal tissue, which may hinder oxygen diffusion from the serosal side. Even if this is the case, our data support the idea that biopsy material may be used to study the response to hypoxia and reoxygenation and to elucidate the cause of the observed R_{TE} reduction.

In summary, this work shows that, at mucolytic concentration, dithiothreitol causes significant, irreversible changes in I_{SC}, V_{TE}, and R_{TE} and therefore should be used with caution in studies in which the preservation of these variables is important; that hypoxia and ouabain cause reductions in I_{SC} and V_{TE}, but while the former decreases R_{TE}, the latter increases it; and that human colon biopsies show, as the isolated mucosa, an asymmetrical response to unilateral hypoxia, larger when it is induced at the serosal side of the epithelium. Therefore, biopsies can be used to study the mechanisms underlying the depressing effects of hypoxia.

Acknowledgments We thank Dr. Dino Sánchez De Simone and Dr. Jorge Izaguirre, both from the Hospital Español, and Prof. Daniel Mathus, from the Hospital Militar, for their kind collaboration for the obtention of biopsy material. Supported by a grant from the Secretaría de Ciencia y Técnica, Universidad Nacional de Cuyo, Mendoza, Argentina.

Conflict of interest None.

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