

Indomethacin reduces short-circuit current and oxygen consumption in normal and chronically hypoxic rat colon

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Summary

Chronic hypobaric hypoxia is a physiological environmental stressor. While its effects on most major organ systems have been extensively studied, few works have addressed hypoxia-induced changes in intestinal transport. The effects of cyclooxygenase blockade with indomethacin on short-circuit current (I_{sc}) and oxygen consumption (QO_2) of the distal colonic epithelium of control rats and rats submitted to hypoxia for 10 days at 0.52 atm were studied. Isolated mucosae were mounted in an Ussing chamber modified for measuring QO_2 while preserving transepithelial vectorial transport. Amiloride was added to the mucosal hemichamber to block a sodium component of I_{sc} present in hypoxic rats. In this condition, basal I_{sc} did not differ between the hypoxic and the control group, but QO_2 was higher in the former. Indomethacin (30 μ mol/L) reduced I_{sc} to the same extent in both groups, but QO_2 reduction was larger in the hypoxic group. Pharmacological blockade of chloride secretion and a low-chloride solution abolished the indomethacin-induced reductions of I_{sc} in both groups, and the reduction of QO_2 in controls, and attenuated but did not suppress the QO_2 reduction in the hypoxic group. Linear regression analysis of QO_2 changes versus I_{sc} changes yielded a significant correlation for both groups, with regression lines with the same slope, but a higher position in hy-

poxic animals. Results suggest that spontaneously released prostaglandins are equally important for maintaining colonic chloride secretion in hypoxic as in normoxic rats, but that, in the former, indomethacin has an additional effect on QO_2 which is unrelated to ion transport.

Index (Key words): Colon, Hypoxia, Indomethacin, Ion transport, Oxygen consumption.

Resumen

La indometacina reduce la corriente de cortocircuito y el consumo de oxígeno en el colon distal de ratas con hipoxia crónica

La hipoxia hipobárica crónica es un estresante ambiental fisiológico. Aunque sus efectos se han estudiado en la mayoría de los sistemas orgánicos, hay pocos trabajos sobre su influencia en el transporte intestinal. Se estudió el efecto del bloqueo de la ciclooxigenasa con indometacina sobre la corriente de cortocircuito (I_{sc}), el consumo de oxígeno (QO_2) del epitelio del colon distal de ratas controles y fueron sometidas a hipoxia durante 10 días a 0,52 atm. Se montaron preparados de mucosa aislada en una cámara de Ussing modificada para medir QO_2 preservando el transporte vectorial transepitelial. Se añadió amilorida a la hemicámara mucosa para bloquear un componente de la I_{sc} debido al sodio presente en ratas hipóxicas. En esta condición, la I_{sc} basal fue similar en ambos grupos, pero el QO_2 fue mayor en los controles. La indometacina (30 μ mol/L) redujo igualmente la I_{sc} en ambos grupos; siendo la disminución de QO_2 mayor en el hipóxico. El bloqueo de la secreción de cloruro (farmacológico y por omisión del ión) suprimió la disminución de I_{sc} en ambos grupos y de QO_2 en el control, y redujo, sin abolir, la disminución de QO_2 en el hipóxico. El análisis de regresión lineal de cambios en QO_2 versus cambios en I_{sc} mostró en ambos

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grupos correlación significativa con líneas de regresión de igual pendiente, pero más alta en el hipóxico. Los resultados sugieren que las prostaglandinas liberadas espontáneamente son igualmente importantes en mantener la secreción de cloruro en ratas hipóxicas y normóxicas, pero en las primeras la indometacina tiene además un efecto depresor del QO_2 no relacionado con el transporte iónico.

Chronic hypobaric hypoxia is an environmental stressor which triggers multiple physiological responses. Many studies have addressed cardiovascular, respiratory, neural, endocrine and metabolic responses to chronic hypobaric hypoxia.^{1,2} There is also a current surge of investigations aimed to understand the molecular biology of adaptation to chronic hypoxia.³ However, the effects of chronic hypoxia on the gastrointestinal tract has been less well explored. The effects of high altitude on the human digestive system was reviewed some time ago.⁴

Several studies on the effect of chronic hypoxia have been performed in the small bowel of experimental animals. It has been reported that chronic normobaric hypoxia modifies small intestinal energy metabolism and disrupts glutathione-dependent detoxicating mechanisms.⁵ Normobaric hypoxia reduced Na,K-ATPase activity and nutrient absorption of the rat jejunum *in situ*.⁶ However, chronic hypobaric hypoxia (10 % O_2 for 10 days) reportedly increases duodenal iron uptake while impairing sodium absorption.⁷

Some effects of chronic hypoxia on the epithelium of the large intestine have been documented. The colonic mucosa of rats submitted to chronic hypoxia has a markedly increased endothelin-1 immunoreactivity in both endothelial and epithelial cells.⁸ *In vitro*, the distal colon epithelium of rats submitted to chronic hypobaric hypoxia has higher baseline transepithelial potential difference (PD), resistivity (Rt), and short-circuit current (Isc) than control rats; however, no evidence of improvement in the tolerance to acute hypoxia was found in the colonic epithelium of chronically hypoxic rats.⁹ In contrast with observations in the small intestine, in the rat colon chronic hypoxia seems to induce an improved coupling between aerobic metabolism measured as oxygen consumption (QO_2) and electrogenic ion transport. Chronic hypobaric hypoxia

is also associated with the expression of amiloride-sensitive sodium absorption despite very low serum aldosterone levels, which may be an adaptive response (Cincunegui et al., *manuscript submitted*).

The processes underlying intestinal electrolyte absorption and secretion are regulated by an interactive network of neural, immune, endocrine and paracrine mechanisms.¹⁰⁻¹³ Neuroimmunoendocrine mechanisms play a role both in physiological adaptive responses and in the pathophysiology of several disorders of electrolyte transport.^{12,14-19} Arachidonic acid derivatives, among them prostaglandins, are key players in mediating epithelial responses to normal and pathological stimuli.^{10,13,14,17,20} Prostaglandins are locally synthesized in the intestinal wall, both by the muscle layer and by the mucosa,^{20,21} where as a rule they inhibit absorption of sodium and chloride, while stimulating chloride and fluid secretion.¹⁰ Prostaglandins are able to influence electrolyte transport both by direct effects on enterocytes, and indirectly through activation of enteric neurons and mast cells.^{10,12,22} Continuous, spontaneous release of prostaglandins seems to stimulate "basal" chloride secretion,^{23,24} while the stimulation of their release contributes to the increased intestinal secretion observed as a response to physiological stimuli like distension^{25,26} and contraction of the muscularis mucosae.²⁷ Enhanced prostaglandin release also plays a role in the epithelial response to a variety of stimulants of intestinal secretion like histamine,²⁸ serotonin,^{18,29,30} neuropeptide Y,³¹ kinins,³²⁻³⁴ cytokines,³⁵⁻³⁷ laxatives,^{38,39} oxidants,^{40,41} morphine withdrawal,⁴² bacterial lipopolysaccharides,⁴³ enterotoxins,^{29,44} lysates of *Entamoeba histolytica*,⁴⁵ *Salmonella* infection,⁴⁶ and cytostatic drugs.⁴⁷

Prostaglandins are synthesized by the cyclooxygenases (COX), a family of enzymes whose molecular biology has recently been reviewed.^{48,49} The COX isozymes 1 and 2 are considered the main targets of non-steroidal antiinflammatory drugs (NSAID),⁵⁰ and cyclooxygenase inhibition plays a prominent role in the gastrointestinal effects of these agents.⁵¹ In the colonic epithelium, inhibition of COX reduces basal and stimulated chloride secretion.^{24,30,46,52,53} Both COX-1 and COX-2 seem to have a role in this epithelium, although their relative contributions are still unclear.^{24,53-55} The NSAID, indomethacin (1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole 3-acetic acid) is an arylalkanoic acid derivative⁵⁶ which non-selectively

tively inhibits both COX-1 and COX-2,^{48,50} and has been widely employed for this purpose in studies of intestinal electrolyte transport.^{22-29,31-39,41-47,57-72}

We have previously shown a significant correlation between Isc and QO_2 under baseline conditions and upon stimulation of chloride secretion with several agents.^{73,74} We were unable to find any report on the effect of indomethacin on electrogenic transport in the colon of chronically hypoxic animals. Moreover, while the effect of indomethacin on non-stimulated distal colon short-circuit current has been documented, its action on epithelial oxygen consumption under conditions which preserve vectorial transport have not been reported. In the present investigation, the effect of non-selective COX blockade with indomethacin on Isc and QO_2 was determined in the isolated colonic mucosa obtained from rats breathing air at normal pressure and rats submitted to chronic hypobaria. Results show that indomethacin reduces Isc and QO_2 in both groups, but in the epithelium of hypoxic animals, the decrease in QO_2 is not completely accounted by the reduction in electrogenic transport.

Methods

Animals. Adult male rats of the Wistar-Hokkaido strain were used. The Committee for Animal Care and Biosafety of our Medical School reviewed and approved the experimental protocol. The animals were fed *ad libitum* on a standard diet for rodents (Cargill Co.) and tap water. All rats were brought from the Medical School animal facility and housed in the same room at 25 °C, with a 12 h light/dark cycle. They were randomly assigned to the control group or to the chronic hypoxia group. Control rats were kept at the ambient pressure of Mendoza (about 92 kPa, or 0.91 atm). Rats submitted to chronic hypoxia were placed for 10 days in individual cages in a 150-L hypobaric chamber with an inner pressure of 51 kPa (about 0.5 atm), roughly equivalent to an altitude of 7,000 m above sea level. The period of chronic hypoxia was selected on the basis of previously reported work (7, 75). The hypobaric chamber was slowly re-pressurized and opened during 30 min each day for cleaning and feeding purposes.

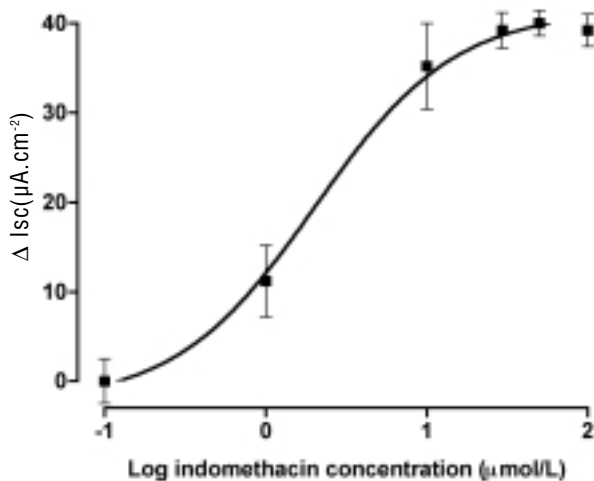
Assessment of systemic response to hypoxia. All animals had their body weight measured at the be-

ginning and at the end of the 10-day period. Food consumption was recorded during the same period. At the time of surgery, trunk blood was withdrawn and treated with sodium heparin for packed red cell volume determination according to a standard microtechnique.

Gas, solutions, and drugs. A mixture of 95% O_2 and 5% CO_2 was employed. Its composition was analyzed and certified by the provider (Air Liquide, Inc.). The Ringer solution had the following composition (in mmol/L): 132.8 Na^+ , 4.5 K^+ , 1.25 Ca^{2+} , 1.00 Mg^{2+} , 114.0 Cl^- , 24.0 HCO_3^- , 1.0 SO_4^{2-} , 0.8 HPO_4^{2-} , 0.2 $H_2PO_4^-$, 10 D (+) glucose. Its osmolality was 280 mOsm/kg H_2O . The composition of the low-chloride solution was (in mmol/L): 136.6 Na^+ , 4.5 K^+ , 1.25 Ca^{2+} , 1.00 Mg^{2+} , 2.5 Cl^- , 24.0 HCO_3^- , 58.1 SO_4^{2-} , 1.6 HPO_4^{2-} , 0.4 $H_2PO_4^-$, 10 D (+) glucose, 93.4 D (+) mannitol. In this solution, sulfate replaced most of the chloride, while mannitol was added to yield the same osmolality of the Ringer solution (280 mOsm/kg H_2O). Both solutions had a pH of 7.40 when gassed with 95% O_2 and 5% CO_2 to saturation, as indicated by a high, steady oxygen concentration.

Amiloride, bumetanide, and indomethacin were purchased from Sigma-Aldrich. Diphenylamine-2-carboxylate was purchased from ICN Flow, and gentamicin from Schering-Plough. Gentamicin was added to the Ringer solution for a final concentration of 91 mg/L to prevent bacterial overgrowth. For each experiment drugs were freshly dissolved in either absolute ethanol (amiloride and diphenylamine-2-carboxylate) or dimethylsulfoxide (bumetanide and indomethacin). Neither ethanol nor dimethylsulfoxide, alone or in combination, had any effect on short-circuit current or oxygen consumption at the added volumes, which were 0.5% or less of the hemichamber volume. Amiloride and diphenylamine-2-carboxylate were added to the mucosal side, as indicated, for a final concentration of 0.1 mmol/L and 1 mmol/L, respectively. Bumetanide was added to the serosal side, as indicated, for a final concentration of 0.1 mmol/L. The use of widely different concentrations of indomethacin, ranging from 1 $\mu\text{mol/L}$ ⁵⁷ to 100 $\mu\text{mol/L}$,²⁴ has been reported in studies of electrogenic intestinal transport. Therefore, to assess the optimal inhibitory concentration a cumulative concentration-response curve was constructed in epithelia from control rats (figura 1).

Figure 1. Cumulative concentration-response for the effect of indomethacin on the reduction of short-circuit current (ΔI_{sc}) in distal colonic epithelium of normoxic rats. Values are mean \pm SEM for $n = 4$.



A concentration of 30 $\mu\text{mol/L}$ was chosen for all experiments, as previously done for both rat and human colonic epithelium.⁷⁶

Surgery, dissection, and mounting. Rats were anaesthetized with diethyl ether. The abdomen was cut open and the colon was dissected from the pelvic brim to the caecum, removed and rinsed free of contents with Ringer solution at 4 °C. A 2.5 to 3-cm segment was cut from the descending colon and a 5-mm diameter polytetrafluoroethylene (Teflon®) rod was inserted into it for mechanical support. The segment was placed in a dissecting bath at 4 °C containing continuously gassed Ringer solution, and stripped free of serosa and submucosal tissue by blunt dissection under a stereomicroscope (Carl Zeiss). The resulting isolated mucosa preparation was cut open along its mesenteric border, gently stretched and the adherent mucus gel layer was carefully removed with a sterile cotton tip soaked in Ringer solution. The mucosa was then mounted as a flat sheet in the Ussing chamber. To minimize variations in electrogenic transport caused by circadian rhythms,⁷⁷ experiments were started between 7.00 AM and 8 AM.

Ussing chamber. The modified Ussing chamber used in the present experiments has been previously described.^{73,74} Each hemichamber had a volume of 21 mL. The chamber was airtight and had an open-

ing of 1 cm^2 . Tight sealing was secured by threaded stoppers and O-rings, which were smeared with silicon grease (Dow Corning 111). Each hemichamber had a bubble trap through which fluid samples may be withdrawn and drugs 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 in both hemichambers. Each hemichamber had a small polytetrafluoroethylene-coated magnetic bar in its bottom, which provided continuous mixing of each hemichamber contents when the chamber was placed on a magnetic stirrer (HI 300N, Hannah Instruments). The chamber had an inbuilt water jacket connected to a water thermostat (VTS13, Radiometer Inc.). The temperature was kept at 37.0 ± 0.5 °C throughout the experiments.

Oxygen consumption determination. Before each experiment, both oxygen meters were calibrated according to the user's manual, and their slopes were checked. Taking into account chamber volume and oxygen solubility in Ringer at 37 °C, QO_2 was calculated from the rate of change in oxygen concentration in both hemichambers. Blankruns performed after each experiment replacing living tissue with a polyethylene membrane showed a rate of decrease in oxygen concentration corresponding to less than 10% of average QO_2 of the unstimulated epithelium.

Electrical measurements. Calomel electrodes connected to each hemichamber through 3% agar in Ringer bridges were used to record transepithelial potential difference. An amplifier with correction for bridge asymmetry and solution resistivity allowed passing current through Ag/AgCl₂ electrodes for clamping the transepithelial potential difference at 0 mV. The experiments were performed with continuous monitoring of the current in digital display and recording in a paper chart recorder under the short-circuit condition, except for brief periodical releases to measure open circuit potential difference. Transepithelial resistivity was calculated from open-circuit potential difference and short-circuit current according to Ohm's law.

Experimental procedures. After the mucosa preparation was mounted and the chamber filled with Ringer solution or low-chloride solution, both hemichambers were gassed with 95% O₂ and 5% CO₂ to

saturation and afterwards closed. A 90-min period was allowed for equilibration,⁷³ after which baseline I_{sc} and QO_2 were measured for 30 min. This was followed by addition of drugs as indicated, and I_{sc} and QO_2 were measured by a second 30-min period. Epithelia with R_t lower than $80 \Omega \cdot \text{cm}^2$ after the equilibration period were discarded. The values of PD, R_t and I_{sc} tabulated represent the averages during the corresponding QO_2 measurement interval.

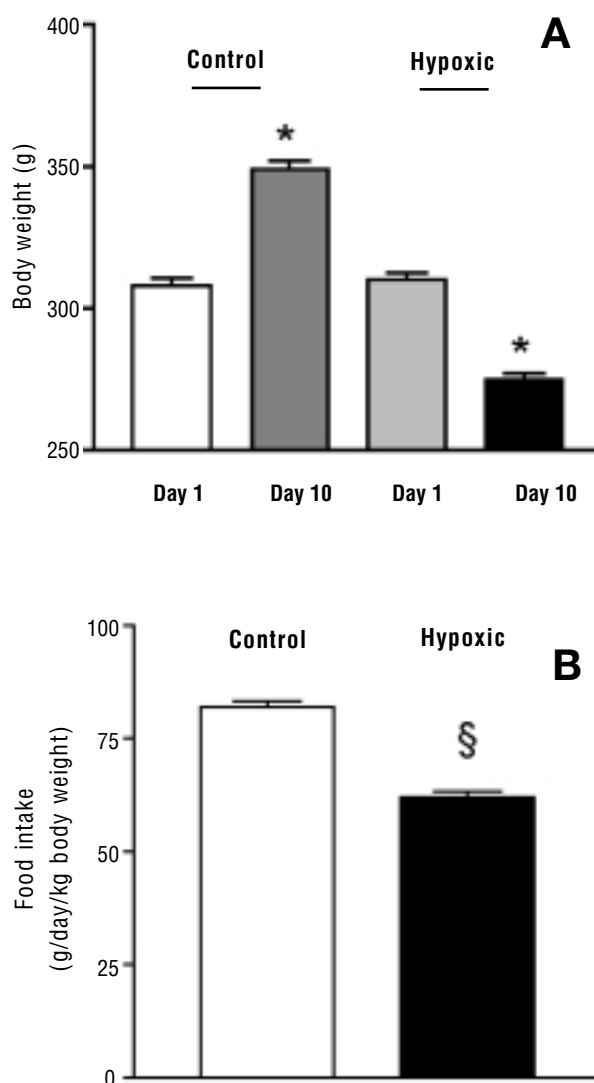
Statistical analysis. A standard commercial software (Prism 3.0 for Windows; GraphPad Software, Inc., San Diego, CA, USA) was employed for statistical analysis of the data. Two-sided Student's *t* tests for paired or non-paired data were used respectively. For comparison of the effect of a treatment in one group a two-sided Student's *t* test for paired data was employed. To compare the effect of the same treatment in different groups, a two-sided Student's *t* test for non-paired data was used. Linear regression analysis, with a check for significant deviation from linearity, was employed for assessment of the relationship between I_{sc} and QO_2 . Unless otherwise stated, results are expressed as mean \pm SEM. Differences were deemed statistically significant at $p < 0.05$.

Results

Systemic response to chronic hypoxia. There was no significant difference in the body weight of rats assigned to each group when they were brought from the School animal house. During the 10-day period, the body weight of control rats ($n = 30$) increased by about 10%, while the body weight of rats submitted to hypoxia ($n = 24$) decreased by about 15% ($p < 0.0001$; figura 2, A). Food intake in the hypoxic group was on average about 20% lower than in the control group ($p < 0.0001$; figura 2, B), resulting in a proportional decrease in sodium intake. In the hypoxic group, water intake was 89 ± 6 mL/day/kg of body weight, while in controls it was 119 ± 5 mL/day/kg of body weight ($p = 0.0004$). At the time of surgery, the packed red cell volume (hematocrit) of control rats was $42.8 \pm 0.7\%$, while that of hypoxic rats was $62.0 \pm 1.1\%$ ($p < 0.0001$).

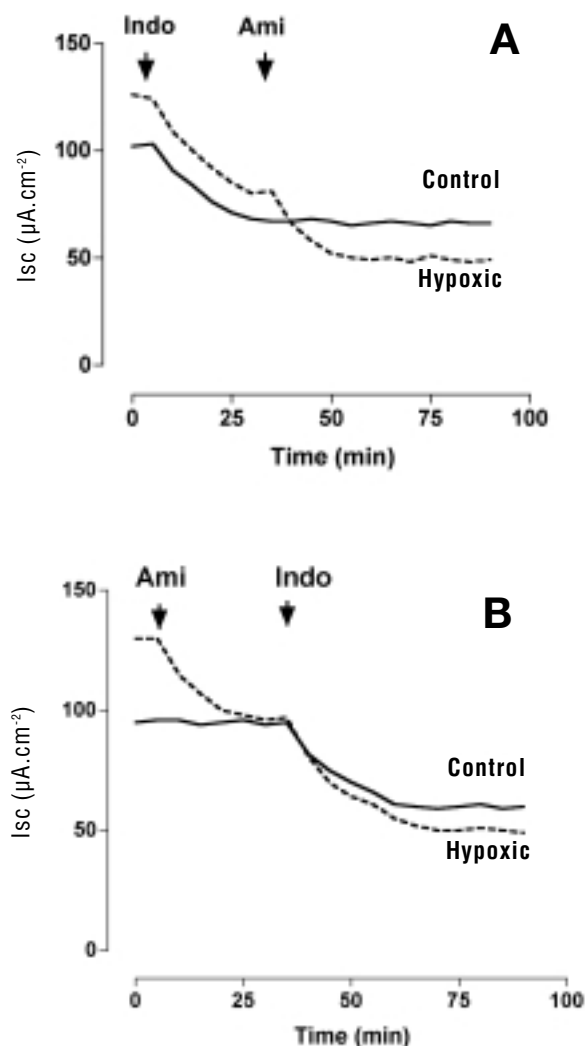
Effect of amiloride. We previously reported that the I_{sc} of the isolated colonic mucosa from chronically hypoxic rats has an amiloride-sensitive component, which is lacking in tissues from controls.⁷⁵ Amiloride reduces I_{sc} and QO_2 in samples from

Figure 2. (A) Changes in body weight in control rats and rats submitted to hypobaric hypoxia (0.52 atm) during 10 days. (B) Food consumption during the 10-day period of control rats and rats submitted to hypobaric hypoxia. Values are means \pm SEM; * $p < 0.0001$ versus day 1; § $p < 0.0001$ versus control.



hypoxic rats but not in those from controls (Cincunegui et al., *manuscript submitted*). In tissues from hypoxic rats, amiloride caused a significant increase in R_t , from $125.5 \pm 3.0 \Omega \cdot \text{cm}^2$ to $129.7 \pm 2.5 \Omega \cdot \text{cm}^2$ ($n = 6$; $p = 0.016$). This increase was not observed in mucosal samples from control rats, in which R_t was $95.3 \pm 2.6 \Omega \cdot \text{cm}^2$ before adding amiloride and $95.2 \pm 3.0 \Omega \cdot \text{cm}^2$ after addition ($n = 6$; $p = 0.905$). As can be seen in figura 3, the effect of

Figure 3. Representative traces of Isc of isolated mucosa preparations from control rats (continuous line) or from hypoxic rats (dashed line), showing the effect of addition of **A**) indomethacin (30 mmol/L) to the serosal hemichamber, followed by amiloride (0,1 mmol/L) to the mucosal hemichamber, and **B**) amiloride (0,1 mmol/L) to the mucosal hemichamber followed by indomethacin (30 mmol/L) to the serosal hemichamber.



indomethacin on the Isc persists both when it is added before amiloride or after it. Since the effect of indomethacin on Isc is due mainly to reduction of chloride secretion,^{23,24,64} all subsequent experiments were performed with previous addition of amiloride (0.1 mmol/L) to the mucosal hemichamber in order to compare the response of both groups to indomethacin.

Effect of indomethacin. Indomethacin increased Rt in samples from both groups. The baseline Rt was $97.7 \pm 2.1 \Omega\cdot\text{cm}^2$ in samples from control rats and $130.3 \pm 3.1 \Omega\cdot\text{cm}^2$ in samples from hypoxic rats ($n = 15$ for each group; $p < 0.0001$). It increased, respectively, to $102.7 \pm 2.4 \Omega\cdot\text{cm}^2$ ($p = 0.011$) and to $135.3 \pm 2.0 \Omega\cdot\text{cm}^2$ ($p = 0.02$) after addition of indomethacin. There was no difference between the Rt increase of both groups (control $5.0 \pm 1.7 \Omega\cdot\text{cm}^2$ versus hypoxic $5.0 \pm 1.9 \Omega\cdot\text{cm}^2$). Baseline Isc did not differ significantly between both groups, but QO_2 was significantly lower in the hypoxic group ($p = 0.022$) because of the blockade of amiloride-sensitive channels (*see above*). Indomethacin reduced Isc and QO_2 in epithelial samples from both groups of rats when the tissues were bathed in Ringer solution (Table 1). There was no significant difference in the reduction of Isc, but the reduction in QO_2 was larger in the hypoxic group ($p = 0.0014$). To assess whether the observed change in QO_2 was accounted by the reduction in Isc in both groups, indomethacin was added to mucosal samples in normal Ringer in which chloride secretion was blocked with bumetanide and diphenylamine-2-carboxylate, and to mucosal samples in low-chloride solution. When chloride secretion was impaired by either procedure, indomethacin reduced neither Isc nor QO_2 in mucosal preparations from control rats. However, in preparations from chronically hypoxic rats, blockade of chloride secretion by either method abolished the change in Isc, but still caused a significant reduction in QO_2 . (Table 2)

Relationship between change in Isc and QO_2 . The reductions in Isc and QO_2 after addition of indomethacin in normal Ringer were analyzed by linear regression, and the results are presented in figura 4. In this figure, reductions in Isc are expressed in $\mu\text{Eq}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}$ to facilitate comparison ($1 \mu\text{Eq}\cdot\text{h}^{-1}\cdot\text{cm}^{-2} = 26.8 \mu\text{A}\cdot\text{cm}^{-2}$). In both groups, a significant correlation between the reduction of Isc and the reduction of QO_2 was found, with correlation coefficients of $r = 0.854$ for the control group and $r = 0.892$ for the hypoxic group. There was no significant difference between the slopes of both lines, but the regression line was significantly higher ($p < 0.0001$) for the samples of the hypoxic group.

Table 1. Effect of indomethacin on short-circuit current and oxygen consumption of rat distal colon epithelial preparations bathed in normal Ringer solution.

| Group | Control (n = 15) | | Chronic Hypoxia (n = 15) | |
|--------------|--|---|--|---|
| | Short-circuit current ($\mu\text{A}\cdot\text{cm}^{-2}$) | Oxygen consumption ($\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}$) | Short-circuit current ($\mu\text{A}\cdot\text{cm}^{-2}$) | Oxygen consumption ($\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}$) |
| Baseline | 73.9 \pm 5.0 | 3.053 \pm 0.119 | 74.9 \pm 3.6 | 2.671 \pm 0.104 |
| Indomethacin | 41.9 \pm 6.9* | 2.591 \pm 0.112* | 35.5 \pm 4.5* | 1.882 \pm 0.087* |

All experiments were carried out in the presence of amiloride (0.1 mmol/L) in the mucosal hemichamber. Indomethacin was added to the serosal hemichamber for a final concentration of 30 $\mu\text{mol/L}$. Data are expressed as mean \pm SEM; * $p < 0.0001$ versus the respective baseline (two-sided Student's t test for paired data); $^{\ddagger} p = 0.003$ versus the reduction in oxygen consumption in control preparations (two-sided Student's t test for non-paired data).

Table 2. Effect of indomethacin on short-circuit current and oxygen consumption of rat distal colon epithelial preparations bathed in normal Ringer solution in the presence of chloride secretion blockers, and in low-chloride solution.

| Group | Control (n = 6 for each treatment) | | Chronic Hypoxia (n = 6 for each treatment) | |
|---|--|---|--|---|
| | Short-circuit current ($\mu\text{A}\cdot\text{cm}^{-2}$) | Oxygen consumption ($\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}$) | Short-circuit current ($\mu\text{A}\cdot\text{cm}^{-2}$) | Oxygen consumption ($\mu\text{mol}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}$) |
| Chloride secretion blockade [§] | | | | |
| Baseline | 12.3 \pm 1.7 | 2.525 \pm 0.111 | 14.2 \pm 1.3 | 2.530 \pm 0.090 |
| Indomethacin | 12.4 \pm 1.6 | 2.500 \pm 0.137 | 13.7 \pm 1.1 | 2.375 \pm 0.088 * |
| Low chloride solution | | | | |
| Baseline | 12.5 \pm 2.2 | 2.477 \pm 0.100 | 11.2 \pm 1.9 | 2.387 \pm 0.079 |
| Indomethacin | 12.7 \pm 1.8 | 2.515 \pm 0.082 | 10.8 \pm 1.6 | 2.227 \pm 0.072 [†] |

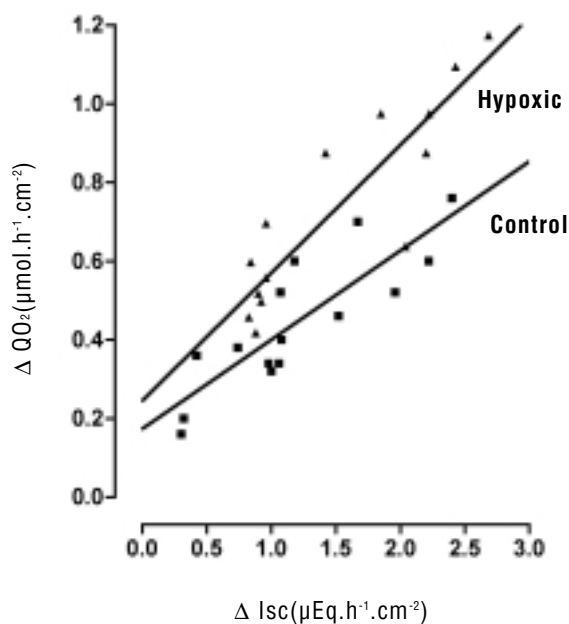
All experiments were carried out in the presence of amiloride (0.1 mmol/L) in the mucosal hemichamber.

Indomethacin was added to the serosal hemichamber for a final concentration of 30 $\mu\text{mol/L}$.

[§] Blockade was accomplished with addition of bumetanide (0.1 mmol/L) and diphenylamine-2- carboxylate (1 mmol/L) to the serosal and mucosal hemichamber, respectively.

Data are expressed as mean \pm SEM; * $p = 0.011$ and [†] $p = 0.007$ versus the respective control (two-sided Student's t test for paired data).

Figure 4. Linear regression of reduction in oxygen consumption (ΔQO_2) as a function of reduction in short circuit current (ΔI_{sc}) caused by indomethacin (30 $\mu\text{mol/L}$) in the isolated colonic mucosa of control rats (squares) and hypoxic rats (triangles); the correlation is significant for both groups, with $r^2 = 0.7297$ and $r^2 = 0.7953$, respectively. The lines do not significantly differ in slope (pooled slope = 0.2782), but the elevation of the line is higher for the hypoxic group ($p < 0.0001$).



Discussion

It is well known that at altitudes above about 3,000 m above sea level both food intake and body weight decrease.⁷⁸ In the rat, transfusion polycythemia does not change the anorexic response.⁷⁹ As previously reported by other authors,⁸⁰ during the experimental period the control rats continued to gain weight, while actually a decrease in body weight was found in hypoxic animals. Although changes in physical activity and energy utilization cannot be ruled out in the present experimental conditions, most of the reduction in body weight can be explained by the hypoxia-induced hypophagia. The increase in packed cell volume indicates an adaptive response to hypoxia,⁸¹ since plasma volume is not decreased⁸² and both plasma sodium and plasma osmolality remain within the normal sea level range.⁷⁵

Amiloride did not reduce I_{sc} or QO_2 in epithelial samples from control rats, but it decreased both va-

riables in tissues from hypoxic rats. However, amiloride did not change the response to indomethacin in either group. To avoid the potential confounding effect of a sodium-dependent I_{sc} component in the hypoxic group, amiloride was routinely added at the apical side of the epithelium. In this condition, there was no significant difference in the mean I_{sc} of both groups, but QO_2 was lower in the hypoxic group. The decrease in I_{sc} upon addition of indomethacin (40 to 50%) did not significantly differ between both groups, suggesting that local production of prostaglandins are equally important in maintaining I_{sc} in the unstimulated epithelium in chronically hypoxic as they are in controls. However, the indomethacin-induced reduction in QO_2 was larger in colonic epithelia from hypoxic rats (about 30% versus 15% in controls). In principle, this difference may be explained by a less efficient coupling between aerobic energy metabolism and electrogenic transport, an effect in QO_2 unrelated to ion transport, or a combination of both. Previous work from our laboratory actually suggested a more efficient coupling between QO_2 and I_{sc} , (Cingunegui et al, *manuscript submitted*). Although in those experiments the amiloride-sensitive component was taken into account, present data do not favor a less efficient coupling either. In the first place, while the regression line between I_{sc} and QO_2 was at a higher level in the hypoxic group, reflecting a larger change in QO_2 for a given change in I_{sc} , its slope did not show a significant difference from that of the control group. This indicates the same proportionality for both groups between the changes in each variable. Second, experiments with the low-chloride solution, whose effects, as previously shown⁸³ are pharmacologically best mimicked by the simultaneous use of two chloride secretion blockers, must be taken into account. In the control group both the decrease in I_{sc} and the reduction in QO_2 caused by indomethacin were completely suppressed by blockade of chloride secretion by both low-chloride solution and pharmacological blockade. In the hypoxic group the change in I_{sc} was equally suppressed, but indomethacin still caused a significant reduction in QO_2 . This difference suggests that in the colonic epithelium of chronically hypoxic rats indomethacin has an additional effect on QO_2 which is not related to the decrease in I_{sc} .

While COX inhibition is responsible for many of

the effects of NSAID, several actions unrelated to COX inhibition have been documented for these drugs.⁸⁴ Some of these actions are relevant, for example, for non-steroidal anti-inflammatory drug-induced enteropathy.^{85,86} In particular, acidic NSAID, like aspirin and indomethacin, are known to have concentration-dependent biphasic effects on cellular respiration, uncoupling oxidative phosphorylation at relatively low concentrations and depressing respiration at higher concentrations.⁸⁷ Indomethacin administered *in vivo* at a dose of 40 mg/kg reduces oxygen uptake of villus enterocytes.⁸⁸ It also decreases oxygen uptake in isolated rat and human jejunal enterocytes,⁸⁹ but at concentrations about 20- to 80-fold higher than that used in the present study. However, the Isc-independent depression of oxygen consumption here reported was only present in the colonic epithelium of hypoxic rats, and chronic hypoxia is known to induce a host of changes in enzymes related to energy metabolism and the respiratory chain.⁹⁰⁻⁹² Thus, it is possible that hypoxia-induced proteomic changes render the epithelium more susceptible to indomethacin-induced respiratory depression.

In summary, results here reported show that indomethacin causes a significantly correlated depression of Isc and QO_2 in the colonic epithelium of both normal and chronically hypoxic rats, but that in the latter there is an additional reduction of QO_2 which may be dependent on hypoxia-induced changes in cell metabolism.

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