

# Pharmacological suppression of rat distal colon chloride secretion requires two blockers

## SUMMARY

Rat distal colon epithelium is frequently employed to assess the effect of natural and synthetic chemicals on chloride secretion. Inhibition of chloride secretion is often reported as the loop diuretic-sensitive portion of short-circuit current ( $I_{sc}$ ). The present work challenges the hypothesis that a loop diuretic alone is able to fully abolish chloride secretion. Isolated mucosa preparations were mounted in an Ussing chamber. The effects on short-circuit current of replacement of normal Ringer by a low (2.5 mmol/L) Cl solution and of blockers of basolateral Na,K,2 Cl symport (bumetanide), apical Cl channels (diphenylamine-2-carboxylate, DPC), and anion exchange (4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid, SITS) alone and combined were assessed. Low Cl reversibly decreased  $I_{sc}$  by 76 %. In normal Ringer, bumetanide decreased  $I_{sc}$  by 65 %. SITS also had a significant effect at the serosal side, but not at the apical side, where DPC caused a 40 % decrease. Chloride replacement, bumetanide and DPC, but not SITS, increased epithelial resistivity. Combined blockade of Na,K, 2 Cl symport and apical Cl channels, of Na,K, 2 Cl symport and anion antiport, or of anion antiport and apical Cl channels was needed to achieve reduction of short circuit current to the same extent seen with chloride replacement. Present results indicate that  $I_{sc}$  of the unstimulated epithelium is mostly due to chloride secretion, and at least two blockers are required to abolish it. This fact should be taken into account in studies of chloride secretion-stimulating agents.

**Index:** Bumetanide, chloride secretion, diphenylamine-2-carboxylate, rat distal colon, short-circuit current, stilbene, Ussing chamber.  
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## INTRODUCTION

The ionic transport properties of rat distal colon have been frequently studied. The rat distal colon is also a common model for probing the effects of neurotransmitters, hormones, autacoids and synthetic chemicals. It is known that, unlike human and rabbit distal coli, short circuit current ( $I_{sc}$ ) in the rat distal colon is insensitive to amiloride. This reflects the fact that in the absence of increased aldosterone levels rat distal colon does not express functional amiloride sensitive sodium channels; most Na absorption takes place

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through an electroneutral mechanism involving apical coupled Na/H and Cl/HCO<sub>3</sub> exchanges<sup>(9)</sup>. However, rat distal colon epithelium generates a  $I_{sc}$  of 60 to 120  $\mu$ A.cm<sup>2</sup>, corresponding to a net electrogenic ion transport of about 2.25 to 5  $\mu$ Eq.h<sup>-1</sup>.cm<sup>-2</sup>.

There is evidence suggesting that this basal  $I_{sc}$  is mainly due to chloride secretion in mucosa-submucosa preparations mounted in Ussing chambers. Both chloride replacement by poorly permeant anions<sup>(2, 16, 21)</sup> and addition of drugs that lower net chloride secretion<sup>(7, 9, 16)</sup> result in decreases in basal  $I_{sc}$ .

According to the currently accepted model of chloride secretion<sup>(1, 13, 18)</sup>, the anion is transported through the basolateral membrane by a Na,K, 2 Cl symport sensitive to furosemide, bumetanide and other loop diuretics. This is a secondary active transport, energized by the sodium electrochemical gradient generated by the basolateral Na,K-ATPase. Chloride then leaves the cell through channels present at the apical membrane, down its electrochemical gradient. These channels are sensitive to blockers like 5-nitro-2-(phenylpropylamino)-benzoate (NPPB) or diphenylamine-2-carboxylate (DPC)<sup>(8, 25)</sup>.

Although bumetanide inhibition of  $I_{sc}$  has been employed as a measure of chloride secretion<sup>(6, 9)</sup>, and both bumetanide and chloride channel blockers have been used to block chloride secretory responses induced by secretagogues<sup>(6, 11, 21, 26, 29, 30)</sup>, we are not aware of investigations assessing the combined effects of both types of chloride secretion inhibitors. Consequently, the present study compared the effect of chloride substitution with that of inhibitors of chloride transport, added either as single agents or sequentially to the isolated mucosa of rat distal colon *in vitro*.

The present study assessed the chloride-dependence of basal  $I_{sc}$  through ion substitution experiments and the use of blockers. It was found that chloride secretion accounts for about 75 % of basal rat distal colon  $I_{sc}$ .

## MATERIAL AND METHODS

**Animals.** Adult male Wistar-Hokkaido rats (BW 250-350 g) were housed and managed according to the Laboratory Animal Care guidelines of our Medical School kept with a 12 h:12 h photoperiod. They drank tap water *ad libitum* and were fed with normal rat chow (Cargill) containing NaCl 0.43 % w/w. Mean daily sodium chloride ingestion was about 360 mg/kg BW.

**Dissection.** Under ether anesthesia, the entire colon was removed, and isolated mucosa preparations were obtained from the late descending colon as previously described<sup>(23)</sup>. Only one preparation was obtained from each animal.

**Electrical measurements.** Isolated mucosa preparations were mounted in an Ussing chamber (opening = 1 cm<sup>2</sup>) thermostated at 37.0  $\pm$  0.5  $^{\circ}$ C. Transepithelial potential difference (PD) was recorded with calomel electrodes connected to the chamber with 3 % Agar-in-Ringer bridges. Ag-AgCl electrodes allowed current to be passed to clamp PD at 0 mV, with correction for bridge asymmetry and compensation for solution resistance. Tissue preparations were studied under short-circuit conditions; the current clamp was released every 5 min to allow open circuit PD to be measured and resistivity (Rt) calculated according to Ohm's law.

**Solutions.** The composition of the solutions is shown in Table I. For the low chloride solution, chloride was almost completely replaced by sulfate, adding mannitol to compensate the difference in osmolality<sup>(19)</sup>. The pH of both solutions was 7.40 when gassed with 95 % O<sub>2</sub>-5 % CO<sub>2</sub>. The bicarbonate-free solution had a pH of 7.40 when gassed with 100 % O<sub>2</sub>. The osmolality of all three solutions was 280 mOsm/kg H<sub>2</sub>O.

**Drugs.** Except for ouabain, drugs were freshly prepared for each experiment. Bumetanide was dissolved in dimethylsulfoxide and added to the serosal side of the Ussing chamber for a final concentration of 0.1 mmol/

**Table 1:** Composition of the solutions employed in the present study. All values are expressed in mmol/L.

Component	Ringer	Low Chloride	Bicarbonate-free
Na+	132.80	136.60	145.3
K+	4.50	4.50	4.50
Ca+	1.25	1.25	1.25
Mg2+	1.00	1.00	1.00
Cl-	114.00	2.50	131.30
HCO3-	24.00	24.00	0.00
HPO42-	0.80	1.60	8.00
H2PO4-	0.20	0.40	2.00
SO42-	1.00	58.10	1.00
D(+) glucose	10.00	10.00	10.00
D(+) mannitol	0.00	93.4	0.00

L. DPC was dissolved in absolute ethanol and added to the apical side for a final concentration of 1 mmol/L. 4-Acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid (SITS) was dissolved in Ringer for a final concentration of 1 mmol/L in the apical or serosal sides. Ouabain was dissolved in isotonic NaCl as a stock solution and serosally added for a final concentration of 1 mmol/L<sup>(22)</sup>. All drugs were purchased from Sigma except for diphenyl-2-carboxylate (ICN).

**Experimental procedures.** After mounting the preparations, 90 min were allowed for equilibration before carrying out solution replacement or addition of drugs. A group of tissues was submitted to replacement of Ringer with the chloride-poor solution, and after a stable  $I_{sc}$  was reached, the latter was replaced again with Ringer. Sham substitutions – i.e, simply replacing the normal Ringer solution bathing the tissue with fresh Ringer solution at the same temperature – were performed in several experiments to rule out an effect of the replacement procedure itself. In some experiments a single drug was tried, while in others several blockers were added. When more than a drug was added, before addition of a second drug a period of stabilization of 20 to 30 min was allowed after the first drug was applied. At the end of all experiments ouabain was added to the serosal side of the chamber.

**Statistics.** Statistical analysis was performed using a standard commercial software (Prism 2.0; GraphPad Software Inc., San Diego, California, USA) by Student's *t* test or one-way analysis of variance followed by Tukey's HSD test, as indicated. Results are reported as mean  $\pm$  SEM. Values of  $P < 0.05$  were considered significant.

## RESULTS

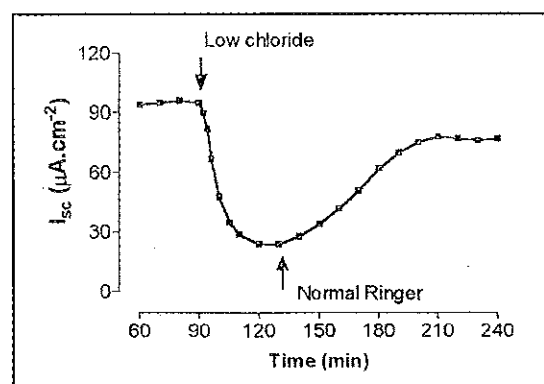
A typical  $I_{sc}$  trace from an actual experiment replacing normal Ringer solution by the low (2.5 mmol/L) chloride solution is shown in Figure 1. Mean  $\pm$  SEM of the change in  $I_{sc}$ , PD and Rt are presented in Table II. In about 20 min  $I_{sc}$  fell to 24 % of control value while Rt was increased by 32 %. The effects were reversible upon switching back to normal Ringer solution. The difference in  $I_{sc}$  before and after low-chloride (which did not reach statistical significance) is readily explained by the time-dependent  $I_{sc}$  decay. Sham substitutions had no effect on  $I_{sc}$ , which was  $103.0 \pm 2.5 \mu\text{A}\cdot\text{cm}^{-2}$  before and  $102.8 \pm 2.7 \mu\text{A}\cdot\text{cm}^{-2}$  after solution replacement ( $n = 5$ ;  $P = 0.847$ ).

The effects of SITS, bumetanide and DPC alone are presented in Table III. SITS added to the apical side of the epithelium had no noticeable effect. In contrast, when added to the basolateral side it caused a 46 % reduc-

tion in  $I_{sc}$  without a significant change in Rt. DPC added to the apical side reduced  $I_{sc}$  and PD by 42 % and 32 %, respectively, with 11 % increase in Rt. Bumetanide added to the serosal hemichamber reduced  $I_{sc}$  and PD by 65 % and 54 %, respectively, and caused a 27 % increase in Rt. Sequential addition of bumetanide and DPC or viceversa caused successive decreases in  $I_{sc}$  as shown in Figure 2. In Table IV mean decreases in  $I_{sc}$  and PD and increases in Rt with bumetanide and DPC are shown. Since the final  $I_{sc}$  values attained by the combined use of bumetanide and DPC did not differ according to the sequence, pooled results are also shown. The effect of the combined basolateral-apical chloride secretion blockade on  $I_{sc}$  was larger than that caused by each individual agent, but less than the sum of both, that is, partially additive. The same was found after sequential addition of bumetanide and SITS to the serosal side of the Ussing chamber, as shown in Fig. 3. The effects of adding bumetanide and SITS to the serosal side or DPC and SITS to the mucosal side of the chamber are presented in Table V.

In bicarbonate-free solution, bumetanide and DPC decreased  $I_{sc}$  and PD when added respectively to the serosal and mucosal sides (Table VI). SITS added to the serosal side caused a fall of both  $I_{sc}$  and PD, sometimes preceded by a transient increase. Addition of bumetanide plus SITS to the serosal hemichamber in the bicarbonate-free solution caused a fractional decrease in  $I_{sc}$  equal to that produced by these agents in normal Ringer, by bumetanide plus DPC and by replacement of normal Ringer by low-chloride solution (Fig. 4). In some experiments bumetanide and DPC were added after chloride removal; no additional effects on  $I_{sc}$  or Rt were observed. Addition of ouabain after chloride removal or the addition of bumetanide and DPC abolished residual  $I_{sc}$  (data not shown).

Figure 1: Typical time course of short-circuit current ( $I_{sc}$ ) upon replacement by low-chloride solution and back to normal Ringer to rat distal colon isolated mucosa.

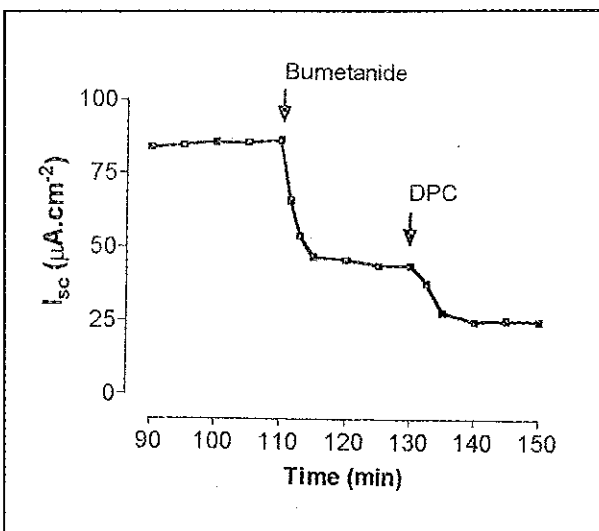


**Table II.** Effect of replacement of normal Ringer ( $[Cl] = 114 \text{ mmol/L}$ ) with low-chloride solution ( $[Cl] = 2.5 \text{ mmol/L}$ ) and back to normal Ringer ( $N = 6$ ). For  $I_{sc}$  and PD A or C vs. B  $P < 0.001$ ; Rt A vs. B  $P < 0.001$ ; B vs C  $P < 0.01$ . Differences between A and C did not reach statistical significance

Solution	Isc ( $\mu\text{A}\cdot\text{cm}^2$ )	PD (mV)	Rt ( $\Omega\cdot\text{cm}^2$ )
A. Normal Ringer	$115.2 \pm 6.8$	$9.8 \pm 0.8$	$85.2 \pm 5.9$
B. Low Chloride	$27.6 \pm 2.2$	$3.1 \pm 0.3$	$112.6 \pm 6.5$
C. Normal Ringer	$95.7 \pm 5.8$	$9.1 \pm 0.7$	$95.1 \pm 6.0$

**Table III.** Effect of bumetanide (0.1 mmol/L), SITS (1 mmol/L) and DPC (1 mmol/L) in the isolated mucosa in Ringer solution. Only one drug was tested in each tissue sample. \*  $P < 0.01$  and #  $P < 0.05$  compared with baseline.

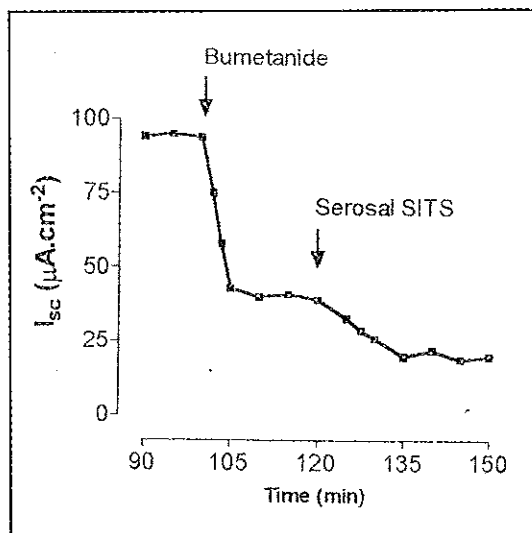
	Isc ( $\mu\text{A}\cdot\text{cm}^2$ )	PD (mV)	Rt ( $\Omega\cdot\text{cm}^2$ )
<b>Bumetanide</b>			
Ringer	$91.0 \pm 6.4$	$8.8 \pm 0.6$	$98.0 \pm 3.9$
+ bumetanide (n = 12)	$32.1 \pm 3.5^*$	$4.0 \pm 0.6^*$	$124.6 \pm 5.2^*$
<b>SITS</b>			
Ringer	$103.4 \pm 4.8$	$12.2 \pm 1.9$	$107.2 \pm 12.9$
+ serosal SITS (n = 9)	$63.9 \pm 6.6^*$	$6.8 \pm 1.5^*$	$102.0 \pm 15.5$
Ringer	$94.9 \pm 7.8$	$10.3 \pm 0.9$	$110.1 \pm 7.1$
+ apical SITS (n = 12)	$96.9 \pm 8.9$	$10.2 \pm 1.0$	$110.2 \pm 8.6$
<b>DPC</b>			
Ringer	$113.5 \pm 9.3$	$10.0 \pm 1.2$	$92.5 \pm 5.5$
+ DPC (n = 9)	$66.3 \pm 7.0^*$	$6.8 \pm 0.9^*$	$102.5 \pm 5.9^*$



**Figure 2:** A trace from a typical experiment showing the effect of successive addition of bumetanide (0.1 mmol/L) and dyphenylamine-2-carboxylate (DPC, 1 mmol/L) to the serosal and mucosal sides of the Ussing chamber, respectively. DPC causes a further reduction of short-circuit current ( $I_{sc}$ ) after bumetanide effect has plateaued.

**Table IV:** Effect of serosal bumetanide (0.1 mmol/L) and apical DPC (1 mmol/L) in the isolated mucosa in normal Ringer solution. Drugs were sequentially added to the appropriate side. \*  $P < 0.01$  and #  $P < 0.05$  compared with baseline.

	Isc ( $\mu\text{A}\cdot\text{cm}^{-2}$ )	PD (mV)	Rt ( $\Omega\cdot\text{cm}^2$ )
<b>Sequence DPC + Bumetanide</b>			
Baseline (n = 6)	113.5 $\pm$ 4.2	10.6 $\pm$ 0.6	92.3 $\pm$ 5.7
DPC	66.3 $\pm$ 4.4*	7.2 $\pm$ 0.4*	108.9 $\pm$ 6.0*
+ Bumetanide	30.2 $\pm$ 2.4	3.9 $\pm$ 0.2	127.7 $\pm$ 7.1
<b>Sequence Bumetanide + DPC</b>			
Baseline (n = 6)	117.0 $\pm$ 3.9	10.9 $\pm$ 0.9	93.3 $\pm$ 6.5
Bumetanide	49.5 $\pm$ 4.1*	5.6 $\pm$ 0.5*	112.7 $\pm$ 7.1
+ DPC	31.0 $\pm$ 3.3	3.9 $\pm$ 0.5	125.8 $\pm$ 8.5
<b>Pooled data (n = 12)</b>			
Baseline	115.2 $\pm$ 2.8	10.7 $\pm$ 0.3	92.8 $\pm$ 4.3
Bumetanide + DPC	30.6 $\pm$ 1.9*	3.9 $\pm$ 0.2*	126.7 $\pm$ 5.5*



*Figure 3:* A representative experiment of the time course of short-circuit current ( $I_{sc}$ ) of the isolated mucosa when bumetanide (0.1 mmol/L) and 4-acetamido-4'-isothiocyantostilbene-2,2'-disulfonic acid (SITS, 1 mmol/L) are successively added to the serosal side of the Ussing chamber. SITS causes an additional depression of  $I_{sc}$  after the effect of bumetanide has reached a steady level.

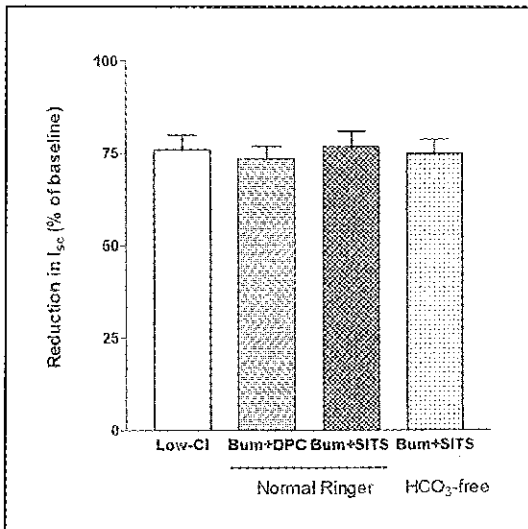
**Table V:** The combined effect of SITS (1 mmol/L) and bumetanide (0.1 mmol/L) added to the serosal side or SITS and DPC (1 mmol/L) added to the mucosal side in the indicated sequences. Isolated mucosa in normal Ringer. \*  $P < 0.001$  vs. Baseline; §  $P < 0.01$  versus the two previous rows.

	Isc ( $\mu\text{A}\cdot\text{cm}^{-2}$ )	PD (mV)	Rt ( $\Omega\cdot\text{cm}^2$ )
<b>Sequence serosal SITS + Bumetanide</b>			
Baseline (n = 6)	87.5 $\pm$ 6.5	7.8 $\pm$ 0.7	90.1 $\pm$ 8.0
SITS	49.0 $\pm$ 3.5*	4.4 $\pm$ 0.3*	90.7 $\pm$ 9.0
+ Bumetanide	18.8 $\pm$ 4.1	1.8 $\pm$ 0.2	99.6 $\pm$ 11.3

	Isc ( $\mu\text{A}\cdot\text{cm}^2$ )	PD (mV)	Rt ( $\Omega\cdot\text{cm}^2$ )
<b>Sequence Bumetanide + serosal SITS</b>			
Baseline (n = 6)	90.1 ± 3.9	9.0 ± 1.2	103.3 ± 16.6
Bumetanide	42.9 ± 7.8*	4.6 ± 0.3*	114.5 ± 22.1
+ SITS	22.4 ± 2.7	2.0 ± 0.2	92.2 ± 14.8
<b>Sequence mucosal SITS + DPC</b>			
Baseline	88.2 ± 5.9	8.4 ± 0.8	94.5 ± 3.5
SITS	90.0 ± 4.6	8.3 ± 0.7	92.7 ± 4.3
SITS + DPC	42.2 ± 3.1	4.8 ± 0.3	115.8 ± 6.2

**Table VI:** The effects of SITS (1 mmol/L) bumetanide (0.1 mmol/L) added to the serosal side or SITS and DPC (1 mmol/L) added to the mucosal side in the indicated sequences to the isolated mucosa in bicarbonate-free solution. \*  $P < 0.001$  and  $^s P < 0.01$  versus the respective baseline.

	Isc ( $\text{mA}\cdot\text{cm}^2$ )	PD (mV)	Rt $\Omega\cdot\text{cm}^2$ )
<b>Serosal SITS</b>			
Baseline (n = 6)	90.5 ± 4.6	8.3 ± 0.5	91.6 ± 4.3
SITS	63.5 ± 4.5	5.8 ± 0.5	91.2 ± 4.2
<b>Serosal Bumetanide + SITS</b>			
Baseline (n = 6)	88.5 ± 3.8	8.7 ± 0.7	97.7 ± 5.1
Bumetanide	22.2 ± 1.6	2.5 ± 0.3	108.3 ± 8.2*
<b>Mucosal DPC</b>			
Baseline (n = 6)	89.1 ± 3.3	8.8 ± 0.7	97.6 ± 4.8
DPC	44.5 ± 2.5	4.8 ± 0.4*	108.1 ± 4.3



**Figure 4:** Comparison of the porcentual change in  $I_{sc}$  caused by low-chloride solution (N = 6), serosal bumetanide (0.1 mmol/L) plus apical dyphenylamine-2-carboxylate (DPC, 1 mmol/L) in normal Ringer (N = 12), and bumetanide plus SITS in normal Ringer (N = 12) and in bicarbonate-free solution (N = 6). No statistically significant difference was found by one-way analysis of variance ( $P = 0.9239$ ).

## DISCUSSION

Certainly a large number of studies in rat distal colon show that secretagogue-induced  $I_{sc}$  increase is suppressed or reduced in chloride-free media<sup>(4, 16, 30)</sup> and by loop diuretics which inhibit the Na,K, 2 Cl symporter present on the basolateral side of the epithelium<sup>(4, 5, 15, 29, 30)</sup>. Addition of epithelial chloride channel blockers like DPC or NPPB, also have been shown to reduce  $I_{sc}$  response to several secretagogues<sup>(11, 27)</sup>. These data plus additional evidence unequivocally show that many agents are able to stimulate electrogenic chloride secretion. However, most of these pharmacological studies report the change in  $I_{sc}$ , without mentioning whether the blockade affects basal  $I_{sc}$ . On the other hand, in some cases stimulated chloride secretion is not completely abolished by loops diuretics<sup>(14, 19, 24)</sup>, or chloride channel blockers<sup>(17)</sup>.

In a paper addressing immune system control of colonic electrolyte ion transport, it was found that basal  $I_{sc}$  of rat colon was chloride-dependent, "probably representing some level of basal electrogenic Cl secretion"<sup>(2)</sup>. A reduction in basal  $I_{sc}$  of about 60 % has been found when chloride is replaced with gluconate<sup>(16, 21)</sup>. Butyrate (25 mM) reduced basal  $I_{sc}$  by 64 %, an effect attributable to a decrease in unidirectional serosa-to-mucosa chloride flux, i.e., in chloride secretion<sup>(7)</sup>. Actually according to the present results basal  $I_{sc}$  is high (60-120  $\mu$ A.cm<sup>-2</sup>) and largely (> 75 %) chloride-dependent. Furthermore, it cannot be completely inhibited by

loop diuretics alone.

Our results show that an amount of  $I_{sc}$  inhibition equivalent to that found in low chloride solution may be obtained with a loop diuretic (bumetanide) plus a chloride-channel blocker (DPC) or, alternatively, a loop diuretic plus SITS. SITS is known to inhibit bicarbonate secretion in rat distal colon<sup>(10)</sup> and in rat colonic crypts, bicarbonate secretion is dependent on chloride secretion<sup>(12)</sup>. However, when added to the basolateral side SITS caused the same reduction in  $I_{sc}$  in normal Ringer and bicarbonate-free solution. In this connection it is worth noting that in human colon, a related stilbene (DIDS) has been reported to decrease cAMP-dependent chloride secretion by inhibition of a basolateral HCO<sub>3</sub>/Cl<sup>-</sup> exchanger. Either a chloride-free solution or bumetanide plus DIDS were required to suppress cAMP-dependent chloride secretion<sup>(20, 28)</sup>. It is possible that a similar mechanism is operative in rat distal colon epithelium, and might help to explain why inhibition of the basolateral Na,K,Cl symport does not always lead to complete block of chloride secretion.

In summary, both experiments with chloride-replacement experiments and with chloride secretion blockers suggest that chloride secretion accounts for about 75 % of basal short-circuit current. In our experiments, in normal Ringer at least two blockers were needed to abolish short-circuit current to the level observed in low-chloride solution. This fact should be taken into account when the chloride-dependence of secretagogue action is pharmacologically assessed.

## Resumen

La mucosa del colon distal de rata es un epitelio modelo que se emplea frecuentemente para evaluar el efecto sobre la secreción de cloruro de sustancias naturales y sintéticas. La inhibición de la secreción de cloruro a menudo se informa como la porción de la corriente de cortocircuito ( $I_{sc}$ ) que es sensible a diuréticos de asa como furosemida o bumetanida. El presente trabajo cuestiona la hipótesis de que un diurético de asa solo sea capaz de abolir completamente la secreción de cloruro. Preparaciones de mucosa aislada se montaron en una cámara de Ussing. Se estudiaron comparativamente los efectos sobre la  $I_{sc}$  del reemplazo de la solución Ringer normal (114 mmol/L de Cl) por una de bajo cloruro (2.5 mmol/L) y de bloqueantes del cotransporte basolateral Na, K, 2Cl (bumetanida; 0.1 mmol/L), de canales apicales de Cl (difenilamina-2-carboxilato, DPC; 1 mmol/L) y del intercambio aniónico (ácido 4-acetamido-4'-isotiocianatostilbano-2,2'-disulfónico, SITS; 1 mmol/L) solos y combinados. La solución pobre en Cl redujo reversiblemente la  $I_{sc}$  en 76 %. En Ringer normal, la bumetanida disminuyó la  $I_{sc}$  en 65 %. El SITS tuvo también un efecto significativo aplicado del lado basolateral, pero no del lado apical, donde por su parte el DPC causó una reducción de 40 %. La reducción del Cl extracelular, la bumetanida y el DPC, pero no el SITS, aumentaron la resistividad transepitelial. Se requirió bloqueo combinado del cotransporte Na, K, 2 Cl y los canales apicales de Cl, o del cotransporte y el intercambiador aniónico basolateral para reducir la  $I_{sc}$  en la misma medida que en medio pobre en cloruro. Los resultados presentes ratifican que la  $I_{sc}$  de la mucosa no estimulada del colon distal de rata es debida principalmente a secreción de cloruro, y demuestra que se requieren dos bloqueantes para abolirla. Este hecho debe tenerse en cuenta en estudios que emplean el bloqueo farmacológico como forma de evaluar el efecto de sustancias que presuntamente promueven la secreción de cloruro.

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