# Mechanism of intestinal secretion. Effect of serotonin on rabbit ileal crypt and villus cells.

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**Research Article**

To determine the mechanism of action of an intestinal secretagogue, serotonin, we have isolated crypt and villus cells and demonstrated Na:H and Cl:HCO3 exchange activity using the intracellular pH-sensitive fluorescent dye, 2,7-bis (carboxy-ethyl)-5,6-carboxy-fluorescein. Serotonin alkalinized both crypt and villus cells. Alkalinization in villus cells was HCO3 dependent and Na independent. In contrast, alkalinization in crypt cells was HCO3 independent and Na dependent. In villus cells, recovery from an alkaline load induced by Cl removal, 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid or propionate pulse, known to occur via the Cl:HCO3 exchange, is inhibited by serotonin. In contrast, in crypt cells, recovery from an acid load induced by Na removal, amiloride and NH4Cl pulse, known to occur via Na:H exchange, is stimulated by serotonin. These data suggest that serotonin is inhibiting Cl:HCO3 exchange in villus cells and stimulating Na:H exchange in crypt cells. These effects of serotonin would be expected to inhibit coupled Na and Cl absorption by villus cells and stimulate HCO3 secretion by crypt cells in the intact ileum.



# Mechanism of Intestinal Secretion

## Effect of Serotonin on Rabbit Ileal Crypt and Villus Cells

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## Abstract

**To determine the mechanism of action of an intestinal secretogogue, serotonin, we have isolated crypt and villus cells and demonstrated Na:H and Cl:$HCO_3$ exchange activity using the intracellular pH-sensitive fluorescent dye, 2,7-bis (carboxyethyl)-5,6-carboxy-fluorescein. Serotonin alkalinized both crypt and villus cells. Alkalinization in villus cells was $HCO_3$ dependent and Na independent. In contrast, alkalinization in crypt cells was $HCO_3$ independent and Na dependent. In villus cells, recovery from an alkaline load induced by Cl removal, 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid or propionate pulse, known to occur via the Cl: $HCO_3$ exchange, is inhibited by serotonin. In contrast, in crypt cells, recovery from an acid load induced by Na removal, amiloride and $NH_4Cl$ pulse, known to occur via Na:H exchange, is stimulated by serotonin. These data suggest that serotonin is inhibiting Cl:$HCO_3$ exchange in villus cells and stimulating Na:H exchange in crypt cells. These effects of serotonin would be expected to inhibit coupled Na and Cl absorption by villus cells and stimulate $HCO_3$ secretion by crypt cells in the intact ileum. (*J. Clin. Invest.* 1991. 87:743–746.) Key words: intestinal secretion • regulation of secretion • intracellular pH • bicarbonate secretion • intestinal secretogogues**

## Introduction

We have recently demonstrated differences in the distribution of transporters between villus and crypt cells in rabbit ileum (1). Villus cells contained Na-glucose and Na-alanine cotransporters and Na:H and Cl:$HCO_3$ exchangers on the brush border membrane (BBM)[1], whereas crypt cells had only a Cl:$HCO_3$ exchanger (Fig. 1). Both cells have a Na:H exchanger on the basolateral membrane (BLM). These results suggest that crypt cells cannot absorb NaCl since this occurs in the ileum by the simultaneous operation of Na:H and Cl:$HCO_3$ exchangers (2). Because of the vectorial arrangement of a Na:H exchanger on the BLM and a Cl:$HCO_3$ exchanger on the BBM, crypt cells may secrete $HCO_3$. These results are consistent with other evidence suggesting that villus cells are primarily absorptive and crypt cells are primarily secretory (3–6). Agents that regulate ileal ion transport may thus affect villus and crypt cells differently. To test this hypothesis, we have determined the effect of a secretogogue, serotonin, on isolated villus and crypt cells.

## Methods

Villus and crypt cells were separated from rabbit ileum by a modification[2] of the calcium-chelation technique of Weiser (7) and maintained in short term culture. Using this method six fractions of cells were sequentially collected and fraction one was used as villus cells and six as crypt cells. Enzyme markers, morphology, and transporter specificity were used to assure good separation of crypt and villus cells. The cells were maintained in short term culture for up to 6–8 h. Viability was assessed by trypan blue exclusion, linear incorporation of leucine into protein, Na-stimulated glucose uptake (villus cells), and preserved cell structure by electron microscopy.[2] The presence of Na:H and Cl:$HCO_3$ exchange and their role in the regulation of intracellular pH has been demonstrated in both cell types.[2]

*Intracellular pH measurements.* The cells were loaded with 10 $\mu$M of the acetoxy methylester of BCECF (2,7-bis [carboxyethyl]-5,6-carboxy-fluorescein) from a 10-mM stock in DMSO for 10 min at 37°C. A cover slip coated with subconfluent monlayer of cells was mounted in a thermostatically controlled cuvette (37°C) in a spectrofluorometer (LS-5; Perkin-Elmer Corp., Norwalk, CT) with constant perfusion to wash away any leaked dye. The dye was alternatively excited at 450 nm and 500 nm, and the fluorescence emission measured at 530 nm. The BCECF fluorescence excitation ratio was calibrated using the high K/nigericin technique.[2] All experiments were performed in $CO_2/HCO_3$ or Na-Hepes($HCO_3$-free) solutions. The standard $CO_2/HCO_3$ solution contained (in mM): NaCl, 115; $NaHCO_3$, 25; $K_2HPO_4$, 2.4; $KH_2PO_4$, 0.4; $MgCl_2$, 1.25; $CaCl_2$, 1.25; and gased with 5% $CO_2$, 95% $O_2$, pH 7.4, at 37°C. The Na-Hepes solution contained (in mM): NaCl, 130; KCl, 4.5; $KH_2PO_4$, 1.2; $MgSO_4$, 1; $CaCl_2$, 1.25; Hepes, 20; and gased with 100% $O_2$, pH 7.4 at 37°C. Chemicals: BCECF-AM was purchased from Molecular Probes Inc., Junction City, OR; 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid and serotonin were purchased from Sigma Chemical Co., St. Louis, MO.

*Data presentation.* Cell viability was assessed after each experiment by trypan blue dye exclusion. Only those experiments in which dye exclusion was > 85% were evaluated. Any given experiment and its control was performed on cells isolated from a single rabbit. A represen-

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1. *Abbreviations used in this paper:* BBM, brush border membrane; BCECF, 2,7-bis (carboxyethyl)-5,6-carboxy-fluorescein; BLM, basolateral membrane; DIDS, 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid.

2. Sundaram, U., R. G. Knickelbein, and J. W. Dobbins. 1990. pH regulation in ileum: Na:H and Cl:$HCO_3$ exchange in isolated crypt and villus cells. *Am. J. Physiol.* In press.

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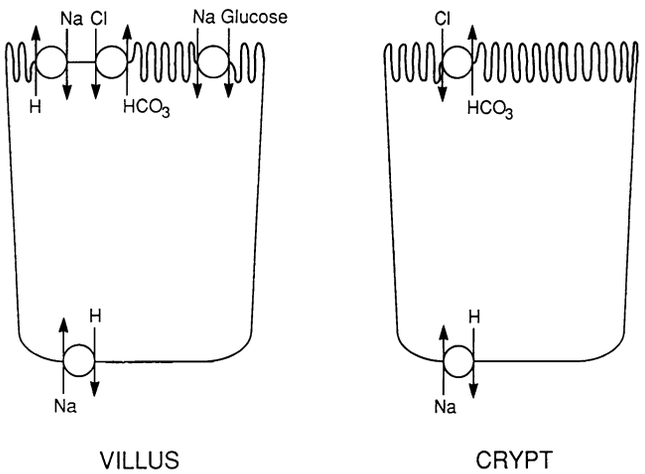


*Figure 1.* A model of crypt and villus cells demonstrating the distribution of some of the transporters.

tative example of each experiment, which was performed on four to six separate cell preparations from different animals, is shown. Paired Student's *t* test was used when statistical analyses were carried out as shown in Results.

## Results

Fig. 2 *A* illustrates that 10 μM serotonin causes a reversible alkalinization of villus cells, but only in the presence of $HCO_3$. Baseline pH increased from 7.21±0.01 to 7.28±0.01 ($n = 6$, $P < 0.01$) in villus cells in the presence of $HCO_3$ whereas there was no change in the absence of $HCO_3$. In contrast, serotonin causes an alkalinization of crypt cells both in the presence and absence of $HCO_3$ (Fig. 3 *A*). In the presence of $HCO_3$ the pH change is from 7.29±0.01 to 7.37±0.01 and in the absence of $HCO_3$ it is from 7.19±0.01 to 7.27±0.01 ($n = 5$, $P < 0.01$). Removal of Na, which acidifies both villus and crypt cells, probably by inhibiting Na:H exchange,[2] does not block serotonin-induced alkalinization in villus cells ($pH_i$ increases from 7.14±0.01 to 7.21±0.01, $n = 4$, $P < 0.01$), suggesting a Na-independent process (Fig. 2 *B*); however, Na removal blocks the effect of serotonin in crypt cells (Fig. 3 *B*).

Removal of Cl or addition of the anion exchange inhibitor DIDS, alkalinizes villus cells and prevents further alkalinization by serotonin (Fig. 2, *C* and *D*). Recovery from the alkaline pH, when Cl is added or DIDS removed, is slower in the presence of serotonin (Fig. 2, *C* and *D*). In Fig. 2 *C* there is no difference between the $pH_i$ of control and 5HT-treated cells (7.38±0.02 vs. 7.38±0.01) just before the readdition of Cl; however, the change in pH in 60 s ($dpH/dt$) after Cl readdition is significantly slower in the presence of 5HT (0.021±0.001 vs. 0.065±0.009, $n = 4$, $P < 0.05$). Similarly, in Fig. 2 *D* there is no difference between $pH_i$ of control and experiment cells (7.41±0.01 vs. 7.41±0.01) just before the removal of DIDS; however, just after DIDS removal $dpH/dt$ is significantly slower in the presence of 5HT (0.040±0.001 vs. 0.078±0.004, $n = 4$, $P < 0.01$). Further, when villus cells are alkalinized by pulsing with Na-propionate the recovery from the alkaline load is slower in serotonin treated villus cells, an effect not seen in crypt cells (Fig. 4). In villus cells, from a maximum pH of 7.63±0.01 in control cells and 7.63±0.01 in 5HT cells, $dpH/dt$ is 0.245±0.008 in control and 0.111±0.014 in 5HT cells ($n = 4$, $P < 0.01$). These results suggest that the alkalinization induced by serotonin results from inhibition of Cl:$HCO_3$ exchange on the BBM of villus cells.

As stated previously, removal of Na blocks the serotonin-induced alkalinization in crypt cells, suggesting a Na-dependent process (Fig. 3 *B*). Amiloride, a Na:H exchange inhibitor, also acidifies the crypt cell and blocks the effect of serotonin (Fig. 3 *C*), suggesting that the alkalinization of crypt cells by serotonin results from stimulation of Na:H exchange. Supporting this possibility, the recovery from the relative acidity induced by Na removal or amiloride is faster in the presence of serotonin (Fig. 3 *B* and *C*). In Fig. 3 *B* there is no difference between $pH_i$ of control and 5HT-treated cells (7.05±0.03 vs. 7.05±0.03) just before Na readdition; however, $dpH/dt$ is significantly faster after Na readdition in the presence of 5HT (0.064±0.003 vs. 0.127±0.015, $n = 4$, $P < 0.05$). Similarly, in

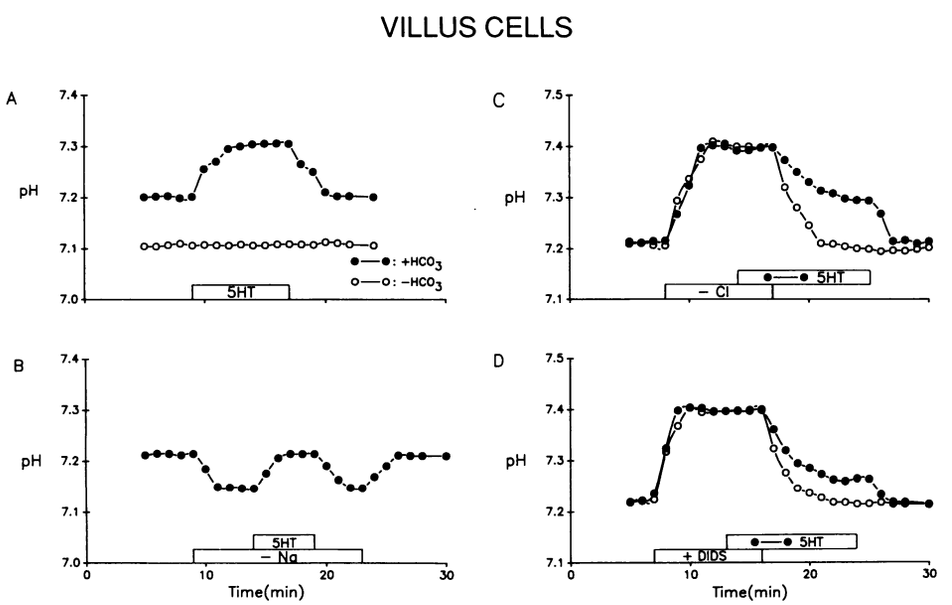


*Figure 2.* Effect of 10 μM serotonin on villus cells. (*A*) Cells were perfused with $HCO_3$-containing (*closed circles*) or $HCO_3$-free (*open circles*) solutions (see below) containing 10 μM serotonin during the period shown. (*B*) Effect of serotonin in Na-free solution in villus cells. Na removal was accomplished by substituting choline for Na in the standard $CO_2/HCO_3$ solution. (*C*) and (*D*) Effect of serotonin on villus cells in Cl-free and DIDS-containing (1 mM) solutions. Cl removal was accomplished by substitution with gluconate in the standard $CO_2/HCO_3$ solution.

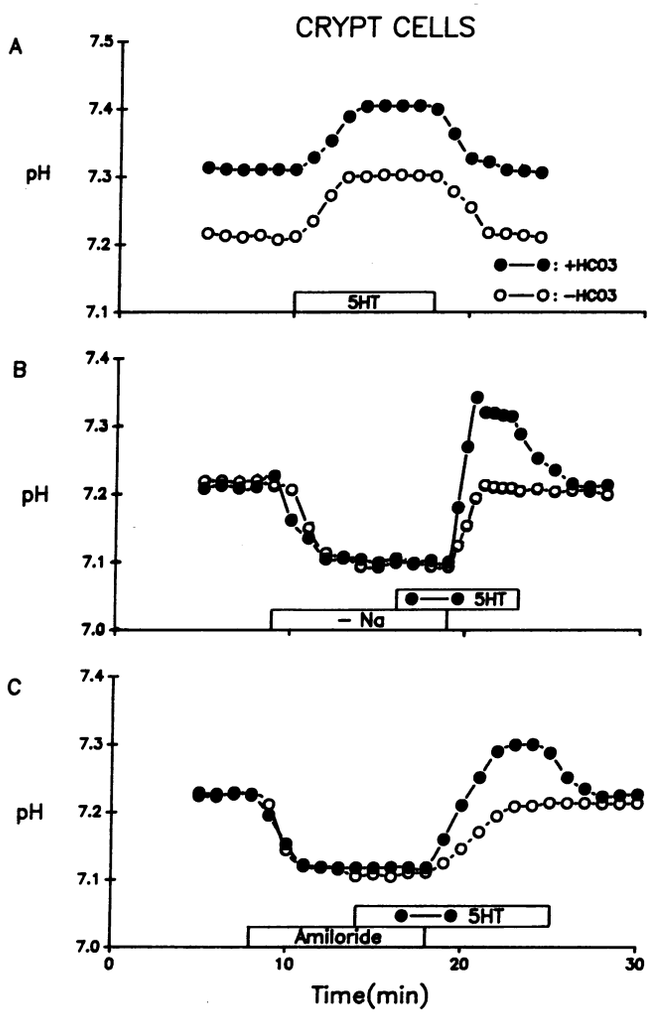


*Figure 3.* Effect of 10 $\mu$M serotonin on crypt cells. (*A*) Cells were perfused with $HCO_3$-containing (*closed circles*) or $HCO_3$-free (*open circles*) solutions containing 10 $\mu$M serotonin during the period shown. (*B*) and (*C*) Effect of serotonin in Na-free and amiloride-containing (1 mM) solutions in crypt cells. Na removal was accomplished by substituting choline for Na.

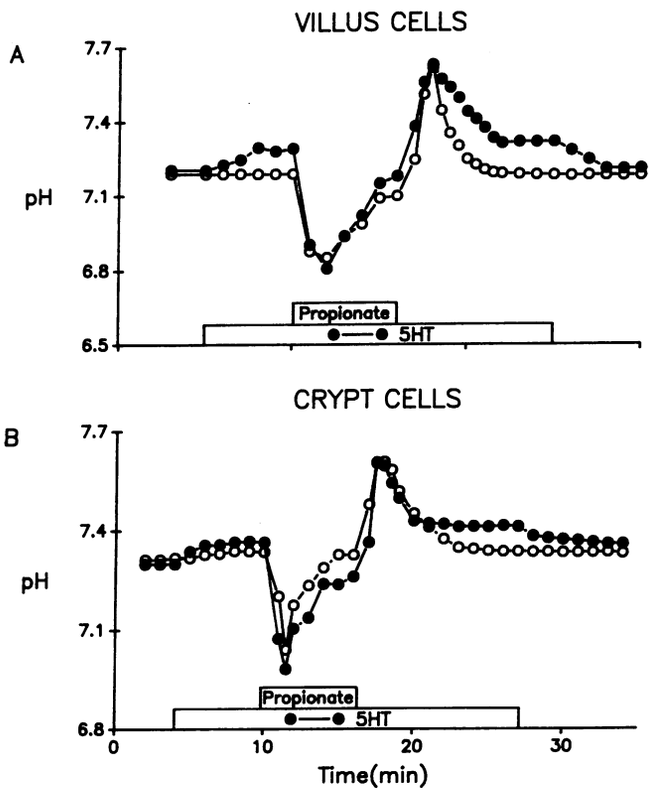


*Figure 4.* Effect of serotonin on recovery from an alkaline load in villus (*A*) and crypt (*B*) cells. The cells are first perfused with the standard $CO_2/HCO_3$ solution then with 50 mM Na-propionate replacing 50 mM of NaCl, in the presence (*closed circles*) or absence (*open circles*) of 10 $\mu$M serotonin during the time period shown.

Fig. 3 *C* there is no difference beween the $pH_i$ of control and 5HT-treated cells (7.09±0.02 vs. 7.10±0.02) just before amiloride removal; however, $dpH/dt$ after amiloride removal is significantly faster in the presence of 5HT (0.022±0.003 vs. 0.041±0.002, $n = 4$, $P < 0.05$). Finally, when crypt cells are acid-loaded by pulsing with $NH_4Cl$, recovery from the acid load is faster in the presence of serotonin in crypt cells, but not villus cells (Fig. 5). In crypt cells, from a nadir pH of 6.59±0.04 in control cells and 6.59±0.03 in experiment cells, $dpH/dt$ is 0.239±0.014 in control and 0.393±0.017 in 5HT cells ($n = 4$, $P < 0.05$).

## Discussion

We have previously demonstrated differences in the distribution of transporters in ileal crypt and villus cells (1) (Fig. 1). Na:H and Cl:$HCO_3$ exchangers are present on the BBM of villus cells, whereas only Cl:$HCO_3$ exchange is present on the BBM of crypt cells. Na:H exchange is present on the BLM of both cell types. This vectorial arrangement of transporters suggests that villus cells absorb NaCl and crypt cells secrete $HCO_3$ (1, 2). Our current studies indicate that serotonin, a neurohumoral agent present in the ileum, has different effects on villus and crypt cells. It induces alkalinization in both cell types, but this appears to result from different mechanisms. In villus cells it is a $HCO_3$-dependent, Na-independent process characterized by a slow recovery from an alkaline load, suggesting inhibition of Cl:$HCO_3$ exchange. The alkalinization induced by Cl removal or DIDS (Fig. 2) suggests operation of the Cl:$HCO_3$ exchanger in the basal state and is consistent with serotonin causing a similar inhibition of Cl:$HCO_3$ exchange in villus cells.

The alkalinization of crypt cells by serotonin, on the other hand, is $HCO_3$ independent, Na dependent, and amiloride sensitive, all suggesting stimulation of Na:H exchange, which is further supported by the serotonin-induced accelerated recovery from an acid load induced by $NH_4Cl$, Na removal, or amiloride.

It has been demonstrated in intact rabbit ileum, in vitro, using the Ussing chamber technique, that serotonin blocks NaCl absorption (8). Our finding that serotonin inhibits Cl:$HCO_3$ exchange in villus cells provides an explanation for this inhibition, since in rabbit ileum Na:Cl absorption occurs by dual operation of Na:H and Cl:$HCO_3$ exchange (2). Donowitz et al. have presented evidence that serotonin's effect in rabbit ileum is mediated by an increase in intracellular Ca that activates protein kinase C (8, 9). Donowitz et al. have also

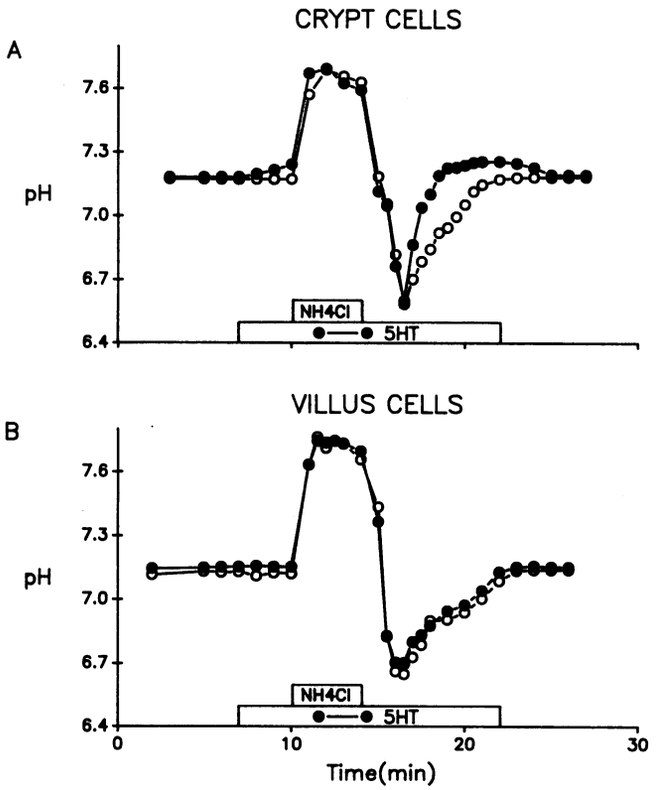


*Figure 5.* Effect of serotonin on recovery from an acid load in crypt (*A*) and villus (*B*) cells. The cells are first perfused with the standard Na-Hepes solution then with 30 mM $NH_4Cl$ replacing 30 mM of NaCl, in the presence (*closed circles*) or in the absence (*open circles*) of 10 $\mu$M serotonin during the time period shown.

recently presented data indicating that protein kinase C activation inhibits Na:H exchange in rabbit ileal BBM vesicles (10). This last observation is clearly not in agreement with our observation of no effect of serotonin on Na:H exchange in villus cells and stimulation of Na:H exchange in crypt cells. There is no obvious explanation for this discrepancy; however, (*a*) serotonin has not been directly shown to activate protein kinase C in ileum; (*b*) differences in technique (brush border vesicles versus isolated cells) may be important; and (*c*) we may have missed a subtle inhibition of Na:H exchange, masked by a greater inhibition of Cl:$HCO_3$ exchange (11).

Hirose and Chang have presented evidence that serotonin inhibits Na:H exchange in chicken enterocytes (12). In this study, however, no attempt was made to distinguish between jejunum and ileum or crypt and villus cells. Further, there are no studies on the effect of serotonin on electrolyte transport in chicken intestine (as opposed to rabbit ileum), thus correlation between isolated cell and intact tissue studies is not possible.

Stimulation of Na:H exchange in crypt cells would be expected to produce OH or $HCO_3$ secretion. This has not been demonstrated with serotonin in intact ileum in vitro. An interesting and as yet unexplained phenomenon is that no secretogogues have been shown to stimulate $HCO_3$ secretion in vitro in ileum, though this clearly has been demonstrated in vivo in humans and animals with cholera enterotoxin (13, 14). Stimulation of Na:H exchange on the BLM of crypt cells is thus a possible explanation for the bicarbonate secretion observed in diarrheal diseases. Secretogogues, including serotonin, stimulate Cl secretion, probably by inserting or activating a Cl channel in the BBM of crypt cells (15). This Cl channel would facilitate $HCO_3$ secretion via Cl:$HCO_3$ exchange by enabling Cl to recycle across the BBM.

Our results not only provide insight into the mechanism of action of serotonin, they further demonstrate the different transport characteristics of villus and crypt cells in the ileum and emphasize the need to separate these two cell populations when determining the regulation of electrolyte transport by neurohumoral agents.

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