Mechanism of Intestinal Absorption
Effect of Clonidine on Rabbit Ileal Villus and Crypt Cells

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Abstract

In intact tissues studies, intestinal absorptogogues stimulate NaCl absorption that occurs via the dual operation of Na:H and Cl:HCO3 exchanges on the brush border membrane (BBM) of villus cells. To determine the cellular mechanism of action of an intestinal absorptogogue, the effect of clonidine was determined on Na:H and Cl:HCO3 exchange in rabbit ileal villus and crypt cells. Using 2,7-bis(carboxyethyl)-5,6-carboxy-fluorescein we have previously shown that recovery from an acid load occurs via Na:H exchange, whereas recovery from an alkaline load occurs via Cl:HCO3 exchange in both cells.

In villus cells, the rate of recovery from a propionate-induced alkaline load was not altered by clonidine. However, clonidine stimulated recovery from an acid load induced by NH4Cl, Na removal, or amiloride. These data suggest that clonidine stimulates Na:H exchange in villus cells.

In crypt cells, the rate of recovery from a propionate-induced alkaline load was also not altered by clonidine. However, in crypt cells, unlike the villus cells, clonidine inhibited recovery from an acid load induced by NH4Cl, Na removal, or amiloride. These data suggest that clonidine inhibits Na:H exchange in crypt cells.

Stimulation of Na:H exchange on the BBM of villus cells would be expected to stimulate coupled NaCl absorption (which occurs by coupling of Na:H and Cl:HCO3 exchange). Inhibition of Na:H in crypt cells, known to be present only on the basolateral membrane, will acidify the cell and may inhibit Cl:HCO3 exchange on the BBM, resulting in the inhibition of HCO3 secretion. (J. Clin. Invest. 1995. 95:2187–2194.) Key words: intestinal absorption and secretion • regulation of secretion • bicarbonate secretion • intestinal absorptogogues • intestinal electrolyte transport

Introduction

In the ileum coupled NaCl absorption occurs via the dual operation of Na:H and Cl:HCO3 exchange on the brush border membrane (BBM) of villus cells. Inhibition of either of these transporters will result in the inhibition of coupled NaCl absorption. In contrast, there is only a Cl:HCO3 exchanger on the BBM of crypt cells, and thus, this cell is not capable of coupled NaCl absorption. However, there is a Na:H exchanger on the basolateral membrane (BLM) of crypt cells and stimulation of this will alkalinize the cell and may stimulate the BBM Cl:HCO3 exchange resulting in HCO3 secretion (1, 2).

We have previously demonstrated that ileal villus and crypt cells respond differently to a given secretogogue. We demonstrated that serotonin inhibited Cl:HCO3 exchange on the BBM of villus cells, whereas it stimulated Na:H exchange on the BLM of crypt cells (3). We have also demonstrated that these two cell types respond differently to agonists that mediate their effects via different intracellular second messengers (3, 4). In these studies we demonstrated that cAMP and Ca inhibit NaCl absorption in villus cells by different mechanisms: cAMP inhibits Na:H exchange, whereas calcium inhibits Cl:HCO3 exchange on the BBM of villus cells. Although both cAMP and calcium inhibit different transporters on the BBM of villus cells, in crypt cells both cAMP and calcium stimulate Na:H exchange that is found only on the BLM. This stimulation, as previously mentioned, may stimulate bicarbonate secretion by the crypt cells (3, 4).

Given these observations with secretagogues, the aim of this study is to determine the mechanism of action of an absorptogogue on these cells. Clonidine, an α2 agonist, known to stimulate NaCl absorption and inhibit bicarbonate secretion in intact tissue studies from the rabbit ileum was used as the absorptogogue (5–8).

Methods

Cell isolation. Villus and crypt cells were separated from rabbit ileum by a calcium-chelation technique previously reported from our laboratory (9) and maintained in short-term culture. Using this method six fractions of cells were sequentially collected. Fraction 1 was used as villus cells and fraction 6 as crypt cells. Several criteria were used to ensure good crypt-villus cell separation, including enzyme markers (alkaline phosphatase for villus and thymidine kinase for crypt), morphology (a well-developed brush border on villus but not crypt cells), transporter specificity (i.e., presence of Na-glucose cotransport in villus but not crypt cells), higher baseline intracellular pH in crypt as compared with villus cells, and higher rates of protein synthesis in crypt as compared with 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 (9). The presence of Na:H and Cl:HCO3 exchange and their role in the regulation of intracellular pH has been demonstrated in both cell types, whereas Na-HCO3 cotransport could not be demonstrated in either cell type (9).

Intracellular pH measurements. Intracellular pH was measured using 2,7-bis(carboxyethyl)-5,6-carboxy-fluorescein (BCECF) as previously described (9). Briefly, the cells were loaded with 10 μM of the
Figure 1. Effect of clonidine on intracellular pH in villus (A) and crypt (B) cells. Cells were perfused with either HCO₃-containing (●, ▲) or HCO₃-free (○, □) solutions. Cells were also perfused with 10 μM clonidine (●, ○) or 10 μM yohimbine followed by clonidine (▲, □) during the period shown.

Results

Fig. 1 demonstrates the effect of clonidine on resting intercellular pH in villus and crypt cells. After achieving a steady state pH, the cells were exposed to clonidine in the presence and absence of HCO₃. Clonidine (10 μM) causes an immediate alkalization of villus cells irrespective of the presence of HCO₃ (Fig. 1 A). Clonidine increased baseline pH from 7.21±0.01 to 7.28±0.01 (n = 4, P < 0.01) in villus cells in the presence of HCO₃ and from 7.10±0.01 to 7.16±0.01 (n = 4, P < 0.01) in the absence of HCO₃. In contrast to the villus cells, clonidine causes an immediate acidification of crypt cells both in the presence and absence of HCO₃ (Fig. 1 B). Clonidine decreased intracellular pH from 7.30±0.01 to 7.25±0.01 (n = 4, P < 0.005) in the presence of HCO₃ and from 7.20±0.01 to 7.14±0.01 (n = 4, P < 0.005) in the absence of HCO₃.

The effects of clonidine are blocked in both villus and crypt cells (Fig. 1) when the cells are first exposed to 10 μM yohimbine, and α₂ antagonist, which by itself has no effect on these cells.

Because the effect of clonidine is HCO₃ independent in both cell types, it is unlikely that the effect of clonidine is on Cl⁻:HCO₃ exchange in either cell type. To confirm that clonidine does not affect Cl⁻:HCO₃ exchange in either cell type, the effect of clonidine on recovery from an alkaline load, known to occur via Cl⁻:HCO₃ exchanger in both cell types (9), was studied. A propionate pulse is used to alkalize both the villus and crypt cells (Fig. 2). In both the villus (Fig. 2 A) and crypt (Fig. 2 B) cells recovery from this alkaline load, known to occur via Cl⁻:HCO₃ exchange, was not altered by clonidine. The initial rate of recovery (dpH/dt) is not affected by clonidine in villus (Fig. 2 C; 0.12±0.01 dpH/min in control and 0.13±0.01 in clonidine, n = 3, P = NS) or crypt cells (Fig. 2 D; 0.06±0.01 dpH/min in control and 0.06±0.01 in clonidine, n = 3, P = NS). These data confirm that clonidine does not affect Cl⁻:HCO₃ exchange in either cell type.

The effect of clonidine on Na⁺:H⁺ exchange in villus and crypt cells was next studied. This was accomplished by determining whether the effect is Na dependent, amiloride sensitive, and by looking at recovery from an acid load that is known to occur via Na⁺:H⁺ exchange in both cells (9). The acid load was induced by three different methods: NH₄Cl pulse, Na removal, and addition of amiloride. Fig. 3 demonstrates the effect of clonidine on recovery from an acid load induced by NH₄Cl. After establishing a steady state pH, cells were briefly pulsed with NH₄Cl, which results in acidification of the villus and crypt cells (Fig. 3). Recovery from this acid load, known to occur via Na⁺:H⁺ exchange in both cells, is accelerated in villus cells (Fig. 3 A) but slowed in crypt cells (Fig. 3 B) by clonidine. In the presence of clonidine, the rate of recovery (dpH/dt) from the acid load...
was faster in villus cells (Fig. 3 C, 0.10±0.01 dpH/min in control and 0.21±0.01 in clonidine, n = 4, P < 0.005). In contrast, in the crypt cells, the rate of recovery from the acid load was slower in the presence of clonidine (Fig. 3 D; 0.08±0.01 dpH/min in control and 0.04±0.01 in clonidine, n = 4, P < 0.001). These data suggest that clonidine stimulates Na-H exchange in villus cells, whereas it inhibits Na-H exchange in crypt cells.

To confirm these observations, the effect of clonidine on recovery from an acid load induced by Na removal was next determined (Fig. 4). Removal of Na acidifies both cells (Fig. 4, A and B) by inhibiting Na-H exchange (9). These maneuvers prevented the alkalization in villus cells and further acidification in crypt cells by clonidine (Fig. 4, A and B) previously seen in baseline conditions (Fig. 1). Thus, these data suggest that the effect of clonidine is Na dependent in both cell types. When Na is re-presented to the villus cells, the rate of recovery from this acid load was faster in the presence of clonidine (Fig. 4 C; 0.12±0.01 dpH/min in control and 0.19±0.01 in clonidine, n = 5, P < 0.001). In contrast, in the crypt cells when Na is re-presented, the rate of recovery from the acid load was slower in the presence of clonidine (Fig. 4 D, 0.06±0.01 dpH/min in control and 0.06±0.01 in clonidine, n = 5, P < 0.001).

To further confirm the observations that clonidine stimulates Na-H exchange in villus cells while inhibiting Na-H exchange in crypt cells, the effect of clonidine on recovery from an acid load induced by amiloride was determined (Fig. 5). Addition of amiloride acidifies both cells (Fig. 5, A and B) by inhibiting Na-H exchange (9). These maneuvers also prevented the alkalization in villus cells and further acidification in crypt cells by clonidine (Fig. 5, A and B) previously seen in baseline conditions (Fig. 1). Thus, these data suggest that the effect of clonidine is amiloride sensitive in both cell types. When amiloride is removed from the villus cells, the rate of recovery from this acid load was faster in the presence of clonidine (Fig. 5 C; 0.07±0.01 dpH/min in control and 0.15±0.01 in clonidine, n = 5, P < 0.001). In contrast, in the crypt cells when amiloride is removed, the rate of recovery from the acid load was slower.
in the presence of clonidine (Fig. 5 D; 0.02±0.01 dpH/min in control and 0.01±0.01 in clonidine, n = 4, P < 0.001). These data also confirm that clonidine stimulates Na:H exchange in villus cells, whereas it inhibits Na:H exchange in crypt cells.

**Discussion**

In the villus cells clonidine increases intercellular pH. This effect of clonidine is bicarbonate independent, Na dependent, amiloride sensitive and is characterized by an accelerated recovery from an acid load induced by three different methods. Recovery from an alkaline load is also not affected. These data are consistent with inhibition of Na:H exchange in crypt cells by clonidine (Fig. 6).

In the crypt cells, unlike the villus cells, clonidine decreases intracellular pH. This effect is also bicarbonate independent, Na dependent, and amiloride sensitive. However, in the crypt cells, unlike the villus cells, the effect of clonidine is characterized by a slowed recovery from an acid load induced by three different methods. Recovery from an alkaline load is also not affected. These data are consistent with inhibition of Na:H exchange in crypt cells by clonidine (Fig. 6).

The effects of clonidine demonstrated in this study are most likely specific for the α2 agonist clonidine. The receptor for this agonist has been demonstrated on rabbit ileal villus and crypt cell membranes (11). Further, yohimbine, an α2 antagonist, blocked the effect of clonidine in both villus and crypt cells (Fig. 1).

It has been previously reported in intact tissue studies from the rabbit ileum that clonidine stimulates coupled NaCl absorption that occurs by the dual operation of Na:H and Cl:\( \text{HCO}_3\) exchange on the BBM of villus cells (12–14). However, the mechanism of stimulation of NaCl absorption by this α2 agonist was not known. This study demonstrates that the mechanism by which clonidine increases coupled NaCl absorption is a result of the stimulation of Na:H exchange in villus cells.

Functionally, two Na:H exchangers have been demonstrated in villus cells (Fig. 6), one each on the BBM and the BLM.
VILLUS CELLS

CRYPT CELLS

Figure 4. Effect of clonidine in Na-free solution and on recovery from an acid load induced by Na removal in villus (A) and crypt (B) cells. The cells were first perfused with the standard Na-Hepes solution and then with Na-free solution (Na removal was accomplished by substitution with tetramethyl ammonium) during the period shown by the bar. Cells were then exposed to and allowed to recover to baseline in the presence (●) or absence (○) of 10 μM clonidine. Clonidine has no effect on villus or crypt cells in the absence of Na. However, with Na re-addition the rate of recovery, dpH/dt, from Na-removal-induced acid load is faster in the presence of clonidine in villus cells (C; 0.12±0.01 dpH/min in control and 0.19±0.01 in clonidine, n = 5, P < 0.001), whereas it is slower in crypt cells (D; 0.06±0.01 dpH/min in control and 0.03±0.01 in clonidine, n = 5, P < 0.001). A and B are representative experiments. In C and D, mean±SEM is shown, and where indicated (●), the dpH/dt at any given time is also significantly different from that of control.

1. With our technique of measuring Na:H exchange activity by monitoring intracellular pH in isolated cells, we cannot tell whether clonidine is stimulating the BBM, the BLM, or both Na:H exchangers. However, because clonidine stimulates coupled NaCl absorption in intact tissue studies from the rabbit ileum, it is likely that clonidine stimulates the BBM Na:H exchange in villus cells (12–14).

Recently Tse et al. (15) have reported the presence of two isoforms of Na:H exchange, NHE2 and NHE3, on the BBM in the rabbit ileum. Our studies do not permit speculation as to which of these isoforms of Na:H exchange is primarily affected by clonidine in the villus cells.

In crypt cells clonidine inhibits Na:H exchange. Knickelbein et al. (1) have demonstrated that only Cl:HCO3 exchange is present on the BBM of crypt cells, whereas Na:H exchange is found on the BLM. Based on the orientation of transporters in these cells, either the direct stimulation of the BBM Cl:HCO3 exchange and/or the stimulation of BLM Na:H exchange, which will alkalinize the cell and may subsequently stimulate the BBM Cl:HCO3 exchange, should result in the secretion of HCO3 by the crypt cells. Conversely, the inhibition of Na:H exchange (i.e., by clonidine in this study) in crypt cells will acidify the cell, which may inhibit the Cl:HCO3 exchange on the BBM, resulting in the inhibition of HCO3 secretion.

Inhibition of HCO3 secretion by α2 agonists has been well described in intact tissue studies from the stomach, duodenum, and ileum (8, 12–14, 16–19). However, in all these tissues the mechanism of HCO3 secretion and the inhibition of this process by α2 agonists is poorly understood. Current evidence would suggest that secretion of HCO3 occurs most likely via Cl:HCO3 exchange in the stomach and duodenum. An ion channel and a paracellular pathway may also be involved in the duodenum. How clonidine inhibits HCO3 secretion in these tissues is not known.
In the ileum, HCO₃⁻ secretion has been well described in vivo and in vitro studies (6). Inhibition of HCO₃⁻ secretion by α₂ agonists has also been described in intact tissue studies (6, 17). However, the mechanism of HCO₃⁻ secretion or the inhibition of this secretion in the ileum is not known.

The recent findings of Minhas and co-workers (20, 21) based on intact tissue studies of the rabbit ileum would support the hypothesis that crypt cells secrete HCO₃⁻. The orientation of transporters in the crypt cell, Cl⁻:HCO₃⁻ exchange on the BBM and Na⁺:H exchange on the BLM (Fig. 6), would also suggest that these cells secrete HCO₃⁻ in the ileum. We have previously demonstrated that two well-known agonists of secretion in the ileum that mediate their action via different intracellular second messengers (calcium and cAMP) stimulate Na⁺:H exchange in crypt cells (3, 4). As previously discussed this stimulation of the BLM Na⁺:H exchange may result in the secretion of HCO₃⁻. Apart from stimulation of BBM Cl⁻:HCO₃⁻ exchange, other possible mechanisms of HCO₃⁻ secretion include a Na⁺:HCO₃⁻ cotransporter or a HCO₃⁻ channel. However, there is presently no evidence for the presence of these later two transport pathways in the rabbit ileum.

In contrast to the effect of secretagogues, clonidine, an absorptagogue, inhibits Na⁺:H exchange in crypt cells. The inhibition of BLM Na⁺:H exchange in crypt cells results in the acidification of these cells, which would subsequently inhibit the BBM Cl⁻:HCO₃⁻ exchange, resulting in the inhibition of HCO₃⁻ secretion, a phenomenon previously unexplained in intact tissue studies.

Thus, the findings of this study and our previous studies suggest that in the ileum HCO₃⁻ transport occurs via Cl⁻:HCO₃⁻ exchanger on the BLM of the crypt cells. Agents mediate HCO₃⁻ secretion and inhibition of secretion by first affecting the BLM Na⁺:H exchanger, which by altering intracellular pH either stimulates or inhibits the BBM Cl⁻:HCO₃⁻ exchange, resulting in the
stimulation or inhibition of HCO₃⁻ secretion by the crypt cell (Fig. 6). This hypothesis is supported by the findings of Minhas and co-workers (20, 21) that crypt cells secrete HCO₃⁻ and the orientation of transporters in the crypt cell (1).

In the crypt cell, the distribution of transporters, Cl⁻:HCO₃⁻ on the BBM and Na⁺:H⁺ on the BLM, may be questioned based on a recent preliminary study by Hoogerwerf et al. (22). In this study they reported that for all three isoforms of Na⁺:H⁺ exchange (NHE1, thought to be on the BLM, and NHE2 and NHE3, thought to be on the BBM in the ileum) are found in villus and crypt cells (22). Inadequate cell separation criteria resulting in the contamination of villus with crypt cells and vice versa could account for this finding. Also, because Knickelbein et al. (1) could not demonstrate Na⁺:H⁺ exchange activity in the BBM of crypt cells, Hoogerwerf et al.'s finding of the message for the various forms of Na⁺:H⁺ exchange needs to be correlated with the presence of functional proteins in BBM and BLM in the crypt cells. Further, Hoogerwerf et al.'s findings are also in contrast to the findings of Bookstein et al. (23). They have, by immunolocalization as well as in situ hybridization, demonstrated that only NHE1 is present in the rat ileal crypt cells, whereas both NHE1 and NHE3 are present in the villus cells. Although this controversy has yet to be settled, it is interesting to note that even if all of the same isoforms of Na⁺:H⁺ exchange are present in villus and crypt cells, they respond markedly different in these cells to not only secretagogues as we previously demonstrated (4) but also to absorptogogues as illustrated in this study.

In conclusion, these studies provide a mechanism for some of the previously unexplained in vivo and in vitro effects of an intestinal absorptogogue on ileal electrolyte transport. Further, because clonidine stimulated Na⁺:H⁺ exchange in villus cells and inhibited Na⁺:H⁺ exchange in crypt cells, these results also emphasize the need to separate these two cell populations in dissecting the regulation of ion transport pathways in the intestine. Not only are the transport pathways different in crypt and villus cells, but the regulation of these transporters by secretagogues and absorptogogues also differs.

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References


