pH stat studies on bicarbonate secretion in the isolated mouse ileum

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ABSTRACT
Bicarbonate secretion occurs in almost all segments of the gastrointestinal tract. This study examined HCO3− secretion in the ileum, since it is less understood than HCO3− secretion in other intestinal segments. Mouse ileal mucosa was mounted in vitro in Ussing chambers, and the mucosal alkalinitzation rate (JOH) was determined by pH stat titration, while the mucosal side was bathed with a buffer-free solution (100% O2) and the serosal side with a HCO3−/CO2-buffered solution. The transmural potential difference (PD) was recorded. The mucosal alkalinitzation rate (JOH) was higher in the presence of mucosal Cl− than in its absence. Forskolin, an activator of adenylate cyclase, enhanced JOH and PD in both the presence and absence of mucosal Cl−. Mucosal SO42− also caused an increase in JOH, although the magnitude was smaller than that induced by Cl−. Mucosal Cl−-dependent JOH was partially inhibited by acetazolamide, 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), tenidap and probably also by niflumic acid, but not by glibenclamide, DIDS or bumetanide. The forskolin-induced JOH value and PD were both inhibited by NPPB and probably also by tenidap. It is concluded that HCO3− secretion in the ileum follows a mucosal Cl−-dependent pathway and a cAMP-activated pathway, each being distinct from each other. The Cl−-dependent pathway is probably mediated by the slc26a6 anion exchanger, and possibly also by the slc26a3 anion exchanger. The cAMP-activated HCO3− secretion is probably mediated by the cystic fibrosis transmembrane conductance regulator.

Bicarbonate (HCO3−) secretion occurs in almost all segments of the gastrointestinal tract and is essential for the physiology and pathophysiology of the gastrointestinal tract (1, 7, 13, 19, 22, 31). In most cases, there are at least two mechanisms for bicarbonate secretion, i.e., a mucosal Cl−-dependent and mucosal Cl−-independent type. It is also known that intestinal HCO3− secretion is stimulated by cAMP (19, 22, 31).

The apical membrane process of the Cl−-independent and cAMP-stimulated secretion is assumed to be mediated by the Cl− channel. The cystic fibrosis transmembrane conductance regulator (CFTR) Cl− channel has been assumed to be one of the Cl− channels responsible for HCO3− secretion (5, 9, 22, 27, 29). CFTR is abundantly expressed in the intestinal epithelia, particularly in the crypt region (2, 8), and is activated by cAMP (24). On the other hand, the apical membrane process of the Cl−-dependent secretion is assumed to be mediated by the Cl−/HCO3− exchanger (5, 13, 14, 19, 22, 25-27, 30, 31). The Cl−/HCO3− exchangers, slc26a3 and slc26a6, have recently been identified and shown to exhibit Cl−/HCO3− exchange activity (3, 4, 12, 15, 18, 20, 34). Both slc26a3 and slc26a6 are expressed throughout the small and large intestines, although slc26a6 expression is higher in the small intestine.
and slc26a3 expression is higher in the large intestine (11, 18, 26, 32). It is therefore likely that these are the Cl / HCO₃⁻ exchangers responsible for Cl⁻-dependent HCO₃⁻ secretion in the intestine.

The purpose of this study is to examine the cellular and membrane mechanisms for HCO₃⁻ secretion in the mouse ileum, since HCO₃⁻ secretion in this segment is not as well understood as that in the other intestinal segments. We specifically examined the involvement of CFTR, slc26a3 and slc26a6 in HCO₃⁻ secretion by determining the effects on HCO₃⁻ secretion of adding several inhibitors and varying the mucosal main anions by using the pH stat method in vitro in Ussing chambers.

MATERIALS AND METHODS

Tissue preparation. The present experiments were performed under the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences recommended by the Physiological Society of Japan. Male mice (ddy; Japan SLC, Shizuoka, Japan) weighing 35–40 g were acclimatized on a standard diet, with food and water being provided ad libitum. The animals were killed by dislocation of the cervical vertebrae, and the ileum was quickly excised. The excised intestinal segment was opened and immersed in an oxygenated HCO₃⁻-buffer solution at room temperature, and the tunica muscularis was removed with fine forceps. The tissue sheets were then mounted vertically between Ussing-type chambers that each provided an exposed area of 0.196 cm². The volume of the bathing solution on each side was 10 mL, and the solution temperature was maintained at 37°C in a water-jacketed reservoir.

Solutions. The mucosal Cl⁻-free solution had the following composition (in mM): Na-glucalone, 140; K-glucalone, 5.4; Ca-(glucalone)₂, 8; Mg-(glucalone)₂, 1.2; and glucose, 10. The mucosal Cl⁻ solution was made by adding 124 mM NaCl in the place of 124 mM Na-glucalone. The mucosal Br⁻, I⁻ and NO₃⁻ solutions were similarly prepared by adding 124 mM Na salt of each anion. The mucosal SO₄²⁻ solution was prepared by adding 62 mM Na₂SO₄ and 62 mM mannitol. The mucosal solutions were gassed with 100% O₂. The serosal HCO₃⁻-buffered solution had the following composition (in mM): NaCl, 119; NaHCO₃, 21; K₂HPO₄, 2.4; KH₂PO₄, 0.6; CaCl₂, 1.2; MgCl₂, 1.2; and glucose, 10. This solution was gassed with 95% O₂/5% CO₂ (pH 7.4). The serosal HCO₃⁻-free, Hepes-buffered solution had the following composition (in mM): NaCl, 114; Na-glucalone, 21; K₂HPO₄, 2.4; KH₂PO₄, 0.6; CaCl₂, 1.2; MgCl₂, 1.2; Hepes, 10; and glucose, 10. The pH value was adjusted to 7.4 with NaOH, and the solution was gassed with 100% O₂.

Measurements of the HCO₃⁻ secretion and transmural potential difference. The alkaline secretion rate (J(HCO₃⁻)) was determined as previously described (6, 13). Briefly, the mucosal solution was continuously titrated to pH 7.2 with 1 mM H₂SO₄ under the automatic control of an HSM-10A pH-stat system (Toa Electronics, Tokyo, Japan). The transmural potential difference (PD) was simultaneously recorded by using a voltage-clamp amplifier (Nihon Kohden, Tokyo, Japan) through paired calomel electrodes, each of which was connected to the bathing solutions with a 1 M KCl-2% agar bridge. Neither the diffusion potential across the tissue nor the change in junction potential generated at the tip of the salt bridges was corrected in the present study. We therefore analyzed only the PD change that was induced by forskolin. Tetrodotoxin (0.3 μM) and indomethacin (10 μM) were added to the serosal solution to minimize the effect of neural tone and endogenous prostaglandins, since neurotransmitters and prostaglandins have been shown to modify the intestinal HCO₃⁻ secretion (22).

Chemicals. We obtained acetazolamide, glibenclamide, 4,4’-diisothiocyanostilben-2,2’-disulphonic acid (DIDS), niflumic acid, 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB), bumetanide and indomethacin from Sigma (St. Louis, MO, USA). Tenidap was generously presented by Pfizer (Groton, CT, USA), and tetrodotoxin was purchased from Calbiochem (Darmstadt, Germany).

Statistics. Each results is the mean±SE (n = no. of animals). Data were compared by using Student’s two-tailed t-test for paired data. Comparisons among three or more groups were made by a one-way analysis of variance (ANOVA) and subsequent Dunnnett post test by using StatView software (SAS Institute, Cary, NC, USA). A value of p < 0.05 is considered significant.

RESULTS

We first explored whether a mucosal Cl⁻-dependent HCO₃⁻ secretion mechanism was present in the mouse ileum. The mucosal alkalinization rate (J(HCO₃⁻)) exhibited a value of 0.71 ± 0.12 μmol × cm⁻² × h⁻¹.
under the mucosal Cl\(^{-}\)-free condition. In the presence of mucosal Cl\(^{-}\), the J\(^{\text{OH}}\) value was significantly higher, with a value of 1.27 ± 0.10 μmol × cm\(^{-2}\) × h\(^{-1}\) (n = 6, Fig. 1A), demonstrating Cl\(^{-}\)-dependent J\(^{\text{OH}}\).

To determine whether this Cl\(^{-}\)-dependent J\(^{\text{OH}}\) reflected the HCO\(_3\)\(^{-}\) secretion, we repeated similar experiments, but under the serosal HCO\(_3\)\(^{-}\)/CO\(_2\)-free, Hepes-buffered condition. The J\(^{\text{OH}}\) values under this condition. In the presence of mucosal Cl\(^{-}\), the J\(^{\text{OH}}\) value was significantly higher, with a value of 1.27 ± 0.10 μmol × cm\(^{-2}\) × h\(^{-1}\) (n = 6, Fig. 1A), demonstrating Cl\(^{-}\)-dependent J\(^{\text{OH}}\).

Fig. 1  Effect of mucosal Cl\(^{-}\) and forskolin on the mucosal alkalinization rate (J\(^{\text{OH}}\)) and transmural potential difference (PD). J\(^{\text{OH}}\) and PD were determined when the mucosal side was bathed with a Cl\(^{-}\) solution (filled symbols) or when bathed with a Cl\(^{-}\)-free (replaced by gluconate) solution (open symbols) using adjacent tissues (n = 6). Forskolin was added at the arrow to the serosal side at a concentration of 10 μM. The serosal side was bathed with (A) a HCO\(_3\)\(^{-}\)/CO\(_2\)-buffered solution, and (B) HCO\(_3\)\(^{-}\)/CO\(_2\)-free, Hepes-buffered solution. (C) Comparison of Cl\(^{-}\)-dependent J\(^{\text{OH}}\) between the HCO\(_3\)\(^{-}\)/CO\(_2\)-buffered and HCO\(_3\)\(^{-}\)/CO\(_2\)-free, Hepes-buffered solutions on the serosal side. Cl\(^{-}\)-dependent J\(^{\text{OH}}\) was calculated as the difference of J\(^{\text{OH}}\) values in the presence and absence of mucosal Cl\(^{-}\). *p < 0.05 (unpaired t-test). (D) Comparison of forskolin-induced J\(^{\text{OH}}\) between the presence and absence of mucosal Cl\(^{-}\) (the serosal HCO\(_3\)\(^{-}\)/CO\(_2\)-buffered condition). *p < 0.05 (paired t-test).
condition were significantly lower both in the absence and presence of mucosal Cl⁻, and the magnitude of the Cl⁻-induced J⁵⁰⁶ ․ increase was significantly smaller than that observed under the serosal HCO₃⁻/CO₂ condition (Fig. 1C). Thus, HCO₃⁻ secretion that was dependent on mucosal Cl⁻ actually occurred in the ileum.

We then explored whether HCO₃⁻ secretion that was activated by cAMP occurred. Forskolin, an activator of adenylate cyclase to increase cAMP, added to the serosal side (10 µM) increased J⁵⁰⁶ ․ both in the absence and presence of mucosal Cl⁻, although the latter increase was significantly smaller than the former (Figs. 1A and 1D). Forskolin-induced J⁵⁰⁶ ․ was completely suppressed under the serosal HCO₃⁻/CO₂-free, Hepes-buffered condition (Fig. 1B). Thus, cAMP-activated HCO₃⁻ secretion actually occurred. Forskolin, in addition, induced increases in PD both in the presence and absence of mucosal Cl⁻, these not being noticeably affected by removing serosal HCO₃⁻/CO₂ (Fig. 1).

In the next series of experiments, we examined the effects of inhibitors on J⁵⁰⁶ ․ to explore the underlying cellular and membrane mechanisms for HCO₃⁻ secretion. We first determined the effect of adding an inhibitor and then of adding forskolin in both the presence and absence of mucosal Cl⁻, the results being summarized in Fig. 2. The carbonic anhydrase inhibitor, acetazolamide (0.1 mM, mucosal), significantly reduced Cl⁻-dependent J⁵⁰⁶ ․ while it did not

Fig. 2 Effect of the inhibitors on J⁵⁰⁶ ․ and PD. The following inhibitors were added to the mucosal side (in µM): acetazolamide (ACZ, 100), glibenclamide (Giben, 100), niflumic acid (NA, 30), NPPB (30), tenidap (10) and DIDS (100). Bumetanide was added to the serosal side at a concentration of 50 µM. n = 6, 6, 6, 6, 5 and 5 for acetazolamide, glibenclamide, niflumic acid, NPPB, tenidap, DIDS and bumetanide, respectively. The result shown in Fig. 1 was adopted as the time control (Cont). Each inhibitor was added after baseline J⁵⁰⁶ ․ had been determined for 30 min. J⁵⁰⁶ ․ in the presence of an inhibitor was determined for 30 min after a stabilizing period of 10–20 min. Forskolin was then added to obtain forskolin-induced J⁵⁰⁶ ․. These measurements were taken in the presence and absence of mucosal Cl⁻ using adjacent tissues. (A) Left panel. Decrease in J⁵⁰⁶ ․ induced by each inhibitor in the absence (−) and presence (+) of mucosal Cl⁻. † and #, significantly different as compared with each time control. *The decrease in J⁵⁰⁶ ․ was significantly different between the presence and absence of mucosal Cl⁻. (A) Right panel. The Cl⁻-dependent J⁵⁰⁶ ․ value was obtained by (J⁵⁰⁶ ․ in the presence of Cl⁻) – (J⁵⁰⁶ ․ in the absence of Cl⁻) and compared before (Basal) and after adding an inhibitor. *p < 0.05. (B) Left panel. Effects of inhibitors on forskolin-induced J⁵⁰⁶ ․ in the absence (−) and presence (+) of mucosal Cl⁻. † and #, significantly different as compared with the control. (B) Right panel. Effects of inhibitors on forskolin-induced PD in the absence (−) and presence (+) of mucosal Cl⁻. † and #, significantly different as compared with the control under each condition.
affect either Cl\textsuperscript{−}-independent J\textsuperscript{OH} or forskolin-induced J\textsuperscript{OH} (or forskolin-induced PD). Therefore, carbonic anhydrase was found to be involved in the Cl\textsuperscript{−}-dependent pathway for HCO\textsubscript{3}\textsuperscript{−} secretion, but not in the Cl\textsuperscript{−}-independent pathway or the cAMP-activated pathway. We then examined the effects of several anion transport inhibitors and found that Cl\textsuperscript{−}-dependent J\textsuperscript{OH} was significantly reduced, but not abolished, by tenidap (30 µM, mucosal) and NPPB (30 µM, mucosal), and possibly also by niflumic acid (10 µM, mucosal). Tenidap in addition significantly decreased the Cl\textsuperscript{−}-independent J\textsuperscript{OH} value. On the other hand, neither glibenclamide (100 µM, mucosal) nor DID (100 µM, mucosal) affected either Cl\textsuperscript{−}-dependent or Cl\textsuperscript{−}-independent J\textsuperscript{OH}. As shown in Fig. 2B, forskolin-induced J\textsuperscript{OH} and PD were significantly inhibited by NPPB, suggesting that cAMP-activated HCO\textsubscript{3}\textsuperscript{−} secretion was mediated by CFTR (see the DISCUSSION section). Finally, we examined the effect of bumetanide (50 µM, serosal), an inhibitor of cAMP-activated Cl\textsuperscript{−} secretion. Bumetanide did not inhibit either basal or forskolin-induced J\textsuperscript{OH}, while it did inhibit forskolin-induced PD as expected. None of inhibitors examined here caused any noticeable change of PD in the absence of mucosal Cl\textsuperscript{−}, while in the presence of Cl\textsuperscript{−}, PD was increased by 0.5–1.5 mV by acetazolamide, niflumic acid and tenidap.

In the final series of experiments, we examined whether such anions as Br\textsuperscript{−}, I\textsuperscript{−}, NO\textsubscript{3}\textsuperscript{−} and SO\textsubscript{4}\textsuperscript{2−} on the mucosal side could stimulate J\textsuperscript{OH} like Cl\textsuperscript{−} did. However, they did not significantly enhance J\textsuperscript{OH} as compared with the effect of gluconate (Fig. 3A). The partial suppression of forskolin-induced J\textsuperscript{OH} by mucosal Cl\textsuperscript{−} shown before was reproduced by all the other anions examined, although only the suppression by I and NO\textsubscript{3}\textsuperscript{−} was statistically significant (Fig. 3B). The forskolin-induced PD increase in the presence of mucosal Br\textsuperscript{−}, I or NO\textsubscript{3}\textsuperscript{−} was, like Cl\textsuperscript{−}, smaller than that in the presence of gluconate, although the forskolin-induced PD increase in the presence of mucosal SO\textsubscript{4}\textsuperscript{2−} was similar to that in the

![Fig. 3](image-url)

Fig. 3  Effect of mucosal Br\textsuperscript{−}, I\textsuperscript{−}, NO\textsubscript{3}\textsuperscript{−} and SO\textsubscript{4}\textsuperscript{2−} on J\textsuperscript{OH}. After J\textsuperscript{OH} had been determined for 30 min in the presence of mucosal Cl\textsuperscript{−}, the mucosal solution was replaced by one containing each anion. J\textsuperscript{OH} was determined again for 30 min after a stabilizing period of 10–20 min. Forskolin was finally added to obtain forskolin-induced J\textsuperscript{OH}. (A) J\textsuperscript{OH} after substituting Cl\textsuperscript{−} with each anion or gluconate. A comparison of the J\textsuperscript{OH} value in the presence of each anion with that in the presence of gluconate did not reach the level of significance. (B) Forskolin-induced J\textsuperscript{OH} in the presence of each anion. †, significantly different from that in the presence of gluconate. (C) Forskolin-induced J\textsuperscript{OH} in the presence of each anion. †, significantly different from that in the presence of gluconate. n = 6 for each anion, except for SO\textsubscript{4}\textsuperscript{2−} for which n = 5.
presence of gluconate (Fig. 3C). It is likely that Cl\(^{-}\), Br\(^{-}\), I\(^{-}\) and NO\(_3\)\(^{-}\), but not SO\(_4^{2-}\) and gluconate, could pass the apical anion channel activated by cAMP, thereby partially cancelling the PD generated by apical Cl\(^{-}\) exit. This channel was probably CFTR, because CFTR can pass Cl\(^{-}\), Br\(^{-}\), I\(^{-}\) and NO\(_3\)\(^{-}\), but not SO\(_4^{2-}\) (10, 16, 24, 28).

Since slc26a6, but not slc26a3, are known to transport SO\(_4^{2-}\) in exchange for HCO\(_3\)\(^{-}\), the stimulation by mucosal SO\(_4^{2-}\) of intestinal HCO\(_3\)\(^{-}\) secretion is an important clue to the involvement of slc26a6 (4, 12, 15, 26, 34). Therefore, we repeated the SO\(_4^{2-}\)-experiment, although determining J\(^{\text{OH}}\) in the presence of mucosal gluconate or SO\(_4^{2-}\) with the same tissue in this case. As shown in Fig. 4, mucosal SO\(_4^{2-}\) slightly but significantly increased J\(^{\text{OH}}\), suggesting that slc26a6 was involved in HCO\(_3\)\(^{-}\) secretion.

**DISCUSSION**

The present study on the isolated mouse ileum has shown that HCO\(_3\)\(^{-}\) secretion occurred by both the mucosal Cl\(^{-}\)-dependent and -independent pathways and that HCO\(_3\)\(^{-}\) secretion was enhanced by cAMP. These findings agree with those from previous studies on the ileum from rabbit and mouse, as well as those on the duodenum and jejunum (19, 22, 27, 29). In addition, we found that the Cl\(^{-}\)-dependent pathway strongly depended on carbonic anhydrase, while the Cl\(^{-}\)-independent pathway and cAMP-activated secretion were both largely independent of the enzyme. The inhibition of Cl\(^{-}\)-dependent HCO\(_3\)\(^{-}\) secretion by a carbonic anhydrase inhibitor has been previously reported in the jejunum (22).

HCO\(_3\)\(^{-}\) secretion through the Cl\(^{-}\)-independent pathway and that through the cAMP-activated pathway exhibited different inhibitor-sensitivity profiles, although both occurred in the absence of mucosal Cl\(^{-}\). The Cl\(^{-}\)-independent HCO\(_3\)\(^{-}\) secretion pathway was inhibited by mucosal tenidap, but was insensitive to mucosal niflumic acid, NPPB and DIDS (Fig. 2A, left panel), while the forskolin-induced HCO\(_3\)\(^{-}\) secretion pathway was inhibited by mucosal NPPB, but was insensitive to mucosal glibenclamide, niflumic acid and DIDS (Fig. 2B, left panel). Therefore, the apical membrane step on the mucosal Cl\(^{-}\}-independent secretion pathway and that on the forskolin-activated secretion pathway are probably distinct from each other. The identity of the molecule responsible for the apical membrane step for mucosal Cl\(^{-}\}-independent HCO\(_3\)\(^{-}\) secretion is not clear, while forskolin-induced HCO\(_3\)\(^{-}\) secretion is probably mediated by apical membrane CFTR. CFTR has been shown to be inhibited by NPPB (17). In addition, the forskolin-induced PD increase, most of which probably reflected Cl\(^{-}\) secretion through the cAMP-activated CFTR Cl\(^{-}\} channel, was also inhibited by NPPB, but not by glibenclamide, niflumic acid or DIDS (Fig. 2B, right panel). It has been reported that cAMP-induced HCO\(_3\)\(^{-}\) secretion in the small intestine is inhibited by NPPB and absent in cystic fibrosis model mice (CFTR-null mice) (5, 9, 22, 27, 29). Glibenclamide is known to inhibit CFTR (17), but had no effect in the present study on either forskolin-induced J\(^{\text{OH}}\) or PD. It is possible that a higher concentration than that used here (0.1 mM) would be required for inhibiting mouse CFTR in native tissue. CFTR is abundantly expressed in all parts of the small intestine (2, 8), and has sufficient permeability to HCO\(_3\)\(^{-}\) (21, 24).

The forskolin-induced HCO\(_3\)\(^{-}\) secretion in the presence of mucosal Cl\(^{-}\) was significantly less than that observed in its absence (Figs 1 and 2). Similar observations have also previously been reported for the rabbit duodenum and ileum and for the rat duodenum (19, 27). The forskolin-induced HCO\(_3\)\(^{-}\) secretion was inhibited not only by mucosal Cl\(^{-}\), but also

![Fig. 4](image-url)

**Fig. 4** Effect of mucosal SO\(_4^{2-}\) on J\(^{\text{OH}}\). The left panel: after J\(^{\text{OH}}\) value in the presence of mucosal gluconate had been determined for 30 min, the mucosal solution had been replaced with the SO\(_4^{2-}\) solution. J\(^{\text{OH}}\) value was determined again for 30 min after a stabilization period of 10–20 min (wavy parallel line). The right panel shows a summary of the results. *Significantly different from gluconate, n = 6.
by mucosal $\Gamma$ and NO$_3^-$, but not by Br$^-$ or SO$_4^{2-}$ (Fig. 3B). One possible explanation for this is that they had an inhibitory effect on HCO$_3^-$ exit through the CFTR channel. The inhibition of HCO$_3^-$ permeability by Cl$^-$ has been previously shown, although the effects of $\Gamma$, NO$_3^-$, Br$^-$ and SO$_4^{2-}$ on the permeability of CFTR to HCO$_3^-$ are not known (23).

It is generally believed that Cl$^-$-dependent HCO$_3^-$ secretion in the intestine is mediated by apical Cl$^-/HCO_3^-$ exchangers. Slc26a3 and slc26a6 Cl$^-/HCO_3^-$ exchangers are expressed both morphologically and functionally in the apical membrane of intestinal epithelial cells, and evidence obtained in the proximal small intestine supports the idea that they are at least in part responsible for Cl$^-$-dependent HCO$_3^-$ secretion (11, 18, 20, 25, 26, 29, 30, 32, 33). The inhibitor-sensitivity profile for Cl$^-$-dependent HCO$_3^-$ secretion was at least in part involved, since SO$_4^{2-}$ could activate HCO$_3^-$ secretion (Fig. 4) suggests that slc26a6 was at least in part involved, since SO$_4^{2-}$ can be transported in exchange for HCO$_3^-$ by slc26a6, but not by slc26a3 in a heterologously expressed system (4, 12, 15, 26, 34). The present results also show that Cl$^-$-dependent HCO$_3^-$ secretion was inhibited by NPPB and tenidap, and also possibly by niflumic acid, but not by DIDS (Fig. 2). Mouse slc26a6 has been shown to be inhibited by DIDS and niflumic acid, although its sensitivity to tenidap, glibenclamide and NPPB has not been reported (4, 12). Therefore, although the absence of inhibition by DIDS is unfavorable and requires further study, we feel that slc26a6 was involved at least partly in Cl$^-$-dependent HCO$_3^-$ secretion in the mouse ileum. The inhibitor-sensitivity profile for Cl$^-$-dependent HCO$_3^-$ secretion observed here also generally agrees with that for slc26a3: mouse and human slc26a3 in a heterologous expression system has been inhibited by tenidap, niflumic acid and NPPB, but was relatively resistant to DIDS at the concentrations used here (3, 15, 18). Therefore, slc26a3 was probably also involved in Cl$^-$-dependent HCO$_3^-$ secretion in the mouse ileum. It cannot be excluded that AE4, another Cl$^-/HCO_3^-$ exchanger which has been shown to be present in the apical membrane of the small intestine, could also be responsible for Cl$^-$-dependent HCO$_3^-$ secretion (35).

In conclusion, the present results suggest that there are at least two HCO$_3^-$ secretion mechanisms at work in the mouse ileum: one is Cl$^-$-dependent HCO$_3^-$ secretion mediated by both slc26a3 and slc26a6, and the other is cAMP-stimulated HCO$_3^-$ secretion mediated by CFTR. These findings are generally in agreement with those reported for the duodenum and jejunum. Further studies are clearly needed to elucidate the molecular mechanisms for and physiological functions of ileal HCO$_3^-$ secretion.

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REFERENCES


