Indirect evidence for cholinergic inhibition of intestinal bicarbonate absorption in humans

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Abstract

Background—The aim of the study was to test the hypothesis that in the fasting state, proximal intestinal HCO₃⁻ absorption, which depends on villus Na⁺/H⁺ exchanger activity, is tonically inhibited by a cholinergic atropine sensitive mechanism.

Subjects—The experiments were performed in 34 healthy volunteers and in eight patients with intestinal villus atrophy.

Methods—HCO₃⁻ absorption was measured with a modified triple lumen perfusion technique in the distal duodenum, the most proximal portion of the small intestine. The study was designed to compensate for the inhibitory effects of atropine on intestinal motor activity.

Results—Atropine had three effects on HCO₃⁻ transport: it reduced HCO₃⁻ concentration at the proximal aspiration site, it displaced the relation between HCO₃⁻ concentration and HCO₃⁻ absorption to the left, and it induced a significant acidification of the perfusate at the distal aspiration site. The magnitude of the stimulatory effect on HCO₃⁻ absorption was similar to the difference between patients with intestinal villus atrophy and healthy controls.

Conclusion—The data suggest that, in the fasting state, duodenal HCO₃⁻ absorption, which depends on villus Na⁺/H⁺ exchanger activity, may be tonically inhibited by an atropine sensitive cholinergic mechanism.

Abbreviations used in this paper: MMC, migrating motor complex; NHE, Na⁺/H⁺ exchanger; PEG, polyethylene glycol.
lumen perfusion technique, we measured distal duodenal HCO₃⁻ absorption in healthy human volunteers under control conditions and after atropine administration. To obtain some additional information about the mechanisms behind the atropine response, we also included a small group of patients with villus atrophy in the study. Motor activity markedly influences HCO₃⁻ absorption in the mixing segment, and to minimise the impact of this factor, the atropine data were compared with periods of similar but “spontaneous” motor quiescence, for which purpose we used the first 30 minutes of the interdigestive motility cycle.

Materials and methods
Thirty four healthy volunteers and eight patients with villus atrophy participated in the study. The healthy volunteers had no history of gastrointestinal disease and were free from medication. The protocol was approved by the ethical committee at Sahlgrenska University Hospital, Göteborg, and all participants had given informed consent. The patients were informed about the scientific nature of the study, and they all participated voluntarily. The study was part of a diagnostic “work up” for patients with suspected coeliac disease, and the patients had agreed to refrain from changes in diet until all diagnostic procedures were completed. The diagnosis of villus atrophy was based on duodenal biopsies in triplicate showing signs of subtotal or total villus atrophy, crypt hyperplasia, and increased numbers of inflammatory cells in the lamina propria. The mean age of the volunteers was 23 (range 20–39) years and the mean age of the villus atrophy patients was 39 (range 20–53) years. The experiments started with the intubation procedure at 7.30 am, and continued for about four hours.

TRIPLE LUMEN PERFUSION TECHNIQUE
This technique has been described in detail in several previous publications to which the reader is referred for technical details. The subjects were intubated with a specially constructed four- or five-lumen nasogastric tube (modified Salem no 12 French, Argyle, Belgium). The tube, which contained one infusion channel, two aspiration channels, and one or two pressure channels, was placed with its tip at the duodenojejunal flexure, with the aid of intermittent fluoroscopy. An isotonic Krebs/ mannitol solution with a bicarbonate concentration of 25 mmol/l (for composition, see below) was infused just distal to the papilla Vateri at a rate of about 6 ml/min, the exact pump rate being calibrated in each experiment. During the early part of the migrating motor complex (MMC) cycle, this solution generates a slightly lumen positive transmural potential difference and a luminal HCO₃⁻ concentration of about 20 mmol/l (or lower) at the entry to the test segment—that is, HCO₃⁻ absorption occurs against an electrochemical gradient (active absorption). In some experiments, the duodenum was instead perfused with saline, to measure secretory HCO₃⁻ fluxes. Both solutions contained polyethylene glycol (PEG, molecular mass 4000 Da) at a concentration of 2 g/l, as a non-absorbable volume marker.

The luminal contents were aspirated at two distal sites, 5 cm (proximal aspiration site) and 15 cm (distal aspiration site) distal to the infusion site—that is, in the mid duodenum and at the level of the duodenoojejunal flexure respectively. The 5 cm segment between the infusion point and the proximal aspiration point acted as mixing segment, and the subsequent 10 cm segment was our test segment. After a run for a period of 40 minutes, fluid was aspirated manually at a rate of 0.7 ml/min from the proximal and distal aspiration sites. The distal aspiration started five minutes after the proximal aspiration, to compensate for estimated mean transit time through the test segment. In most experiments, aspirates were collected at 15 minute intervals, and mean values from two 15 minute aspiration periods were used as 30 minute data. In some experiments (mainly the villus atrophy group), fluid was instead collected in 30 minute aliquots.

ANALYSIS OF CONCENTRATIONS OF PEG AND HCO₃⁻ IN THE PROXIMAL AND DISTAL ASPIRATE
The concentration of PEG was determined by the turbidimetric method of Hyden. For the analysis of HCO₃⁻ concentration, 2 ml samples of mixed aspirates were collected in closed plastic vials. pH and PCO₂ of the aspirate were measured in an automatic acid-base analyser (ABL 50; Radiometer, Copenhagen, Denmark), and HCO₃⁻ concentration [HCO₃⁻] was calculated from Henderson-Hasselbalch’s equation: $[\text{HCO}_3^-] = S \times [\text{PCO}_2] \times 10^{-\text{pH}−\text{pKa}}$, where $S$, the solubility constant for CO₂, has a value of 0.23 mmol/kPa per litre at 37°C and the value for pKa, the first dissociation constant of carbonic acid in a plasma-like solution, is 6.10⁻³.

CALCULATIONS OF HCO₃⁻ ABSORPTION
HCO₃⁻ absorption in the test segment was calculated from the following formula: $Q_{pr} − 0.7 = \frac{[\text{HCO}_3^-]_{pr} − [\text{HCO}_3^-]_{di}}{[\text{PEG}]_{pr}/[\text{PEG}]_{di}}$, where $Q_{pr}$ = volume flow at the proximal aspiration site (= Q × [PEG]_{pr}/[PEG]_{di}), Q = pump rate (= 6 ml/min), [PEG] = PEG concentration, 0.7 = proximal aspiration rate (ml/min), NFT = net fluid transport within the test segment (= Q_{pr} − 0.7) × (1 − [PEG]_{pr}/[PEG]_{di}). Subscripts pr, di denote infusion and proximal and distal aspiration sites respectively.

RECORDING OF DUODENAL MOTOR ACTIVITY
A modified Arndorfer system was used to record duodenal motility. Motor activity was always recorded 2 cm distal to the infusion site—that is, in the descending duodenum distal to the papilla Vateri, and in most experiments also at the duodenojejunal flexure, to confirm propagation of the activity front (phase III) of the MMC. The thin polyethylene catheters used for pressure recording were perfused with isotonic saline via narrow capillaries at a rate of 0.3 ml/min, and inflow pressure was recorded by pressure transducers connected to
Intestinal bicarbonate absorption

In an unsaturated system, $\text{HCO}_3^-$ equilibration with 5% CO$_2$ and the osmolality of the solution was approximately 8.0 (7.4 after attained PEG at a concentration of 2 g/l. The pH in Louis, Missouri, USA). The solution also contained 4.7 KCl, 1.2 KH$_2$PO$_4$, 25 NaHCO$_3$, 1.2 MgCl$_2$, and 30 mannitol (all from Sigma Chemicals, St

RESULTS

CONTROL PERIODS AND CORRECTIONS FOR EFFECTS OF ATROPINE ON MOTOR ACTIVITY

As atropine reduces motor activity, we compared periods of “spontaneous” motor quiescence in the other groups (controls, NaCl, villus atrophy) with the atropine data. This was achieved by measuring during the first 30 minutes after phase III of the MMC. Phase III of the MMC cycle was defined as a period of motor activity at slow wave frequency (10–12 contractions/min) with a duration of at least two minutes. If pressure was recorded at two sites, propagation of the front was also included in the definition of phase III activity.

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EXPERIMENTAL GROUPS

The control group (n = 11) consisted of healthy volunteers, perfused with Krebs/manitol and receiving no experimental intervention. The saline group (n = 10) were also healthy volunteers perfused with saline and receiving no experimental intervention. The atropine group (n = 13) were healthy volunteers perfused with Krebs/manitol and receiving atropine. Atropine (Kabi Vitrum AB, Stockholm, Sweden; 0.5 mg/ml) was given intravenously at a dose of 0.01 mg/kg 30–45 minutes after a phase III period, and this dose was followed by two additional injections at 30 minute intervals (0.1 mg each). The “atropine” data were mean values obtained during the 90 minutes after atropine injection. The villus atrophy group (n = 8) consisted of patients with villus atrophy due to untreated coeliac disease; they were perfused with Krebs/manitol and received no experimental intervention.

INTESTINAL PERFUSATES

In most experiments the intestine was perfused with a Krebs/manitol solution with the following composition (in mmol/l): 122 NaCl, 4.7 KCl, 1.2 KH$_2$PO$_4$, 25 NaHCO$_3$, 1.2 MgCl$_2$, and 30 mannitol (all from Sigma Chemicals, St Louis, Missouri, USA). The solution also contained PEG at a concentration of 2 g/l. The pH of the solution was approximately 8.0 (7.4 after equilibration with 5% CO$_2$) and the osmolality approximately 310 mosmol/kg. In the saline group, we perfused the intestine with physiological saline (NaCl 154 mmol/l), to which was added PEG 2 g/l. Krebs/manitol was used as perfusate in the villus atrophy group.

CALCULATIONS AND STATISTICAL EVALUATION

In an unsaturated system, $\text{HCO}_3^-$ absorption will be concentration dependent. Luminal $\text{HCO}_3^-$ concentration at the entry to the test segment was not significantly different in the control and villus atrophy groups ($\approx$ 20 mmol/l), but was about 20% lower in the atropine group (15.2 (0.9) mmol/l; p<0.01 versus control and villus atrophy groups; results are mean (SE)).

Figure 2 illustrates the relation between luminal concentration and absorption of $\text{HCO}_3^-$ in the control group. During perfusion with the $\text{HCO}_3^-$-containing solution (filled triangles), there was a significant linear correlation between $\text{HCO}_3^-$ absorption and $\text{HCO}_3^-$ concentration measured at the proximal aspiration site ($r = 0.80$, p<0.01). During perfusion
Table 1  Duodenal motor activity and luminal HCO₃⁻ concentration at entry to test segment, in the different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Motor activity (contractions/min)</th>
<th>HCO₃⁻ concentration (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 11)</td>
<td>0.9 (0.2)</td>
<td>21.4 (1.4)</td>
</tr>
<tr>
<td>NaCl (n = 10)</td>
<td>1.0 (0.2)</td>
<td>1.6 (0.8)***</td>
</tr>
<tr>
<td>Atropine (n = 13)</td>
<td>1.0 (0.1)</td>
<td>15.2 (0.9)**</td>
</tr>
<tr>
<td>Villus atrophy (n = 8)</td>
<td>1.0 (0.2)</td>
<td>19.8 (1.1)</td>
</tr>
</tbody>
</table>

Values are mean (SE) for the number of experiments given in parentheses. **p<0.01; ***p<0.001 versus controls.

Figure 2  Duodenal HCO₃⁻ absorption as a function of luminal HCO₃⁻ concentration at the entry to the test segment, in healthy controls. All data emanate from the first 30 minutes of the migrating motor complex cycle (low motor activity). Filled triangles indicate Krebs/mannitol perfused segments and open triangles saline perfused segments. HCO₃⁻ transport is expressed as mmol/min per 10 cm; positive values denote net absorption. The equation of the regression line for the Krebs/mannitol perfused segment is also given in the figure.

Figure 3  Relation between luminal HCO₃⁻ concentration and net HCO₃⁻ absorption in atropine treated subjects. A total of 10 of 13 subjects (solid squares) followed a regression line (solid line) that was almost exactly parallel to the control regression line (dashed line). The three remaining subjects (open squares) were on the control line. The equation of the regression line for the 10 subjects is given in the figure.

Figure 4  Calculated net HCO₃⁻ absorption at a luminal HCO₃⁻ concentration of 20 mmol/l in the three groups. Control data and atropine data were recalculated from the slope of the regression line in figs 2 and 3. Villus atrophy data were measured data (mean luminal HCO₃⁻ concentration 19.8 (1.1) mmol/l). Values are mean (SE). Asterisks above bars indicate a significant difference from control values. A significant difference between villus atrophy and atropine data is indicated separately. Significance levels were: *p<0.05; **p<0.01; ***p<0.001. For further explanations, please see the text.

With HCO₃⁻-free solution (NaCl, open triangles), HCO₃⁻ transport was not significantly different from zero.

Villus atrophy patients, there was no consistent relation at all between luminal HCO₃⁻ concentration and absorption (r = 0.075, p = 0.85; data not shown), and therefore this mode of recalculation was not considered appropriate for this group. However, luminal HCO₃⁻ concentration at the proximal aspiration site in the villus atrophy patients was not significantly different from that in the control group (19.8 (1.1) vs 21.4 (1.4) mmol/l). To be able to directly compare absolute HCO₃⁻ absorption values in all three groups at a similar luminal HCO₃⁻ concentration, we therefore used actual measured data from the control and villus atrophy groups and mathematically normalised the atropine data to the same luminal concentration (20 mmol/l), from the slope of the (control and atropine) regression lines. Figure 4 shows the results of this calculation. In the control group, the normalised HCO₃⁻ absorption rate was 35.4 (3.8) µmol/min per 10 cm, which increased to 48.0 (3.4) µmol/min per 10 cm after atropine administration (p<0.05 vs control data). In the villus atrophy group, the normalised HCO₃⁻ absorption rate was 15.2 (5.7) µmol/min per 10 cm (p = 0.01 vs controls, p<0.001 vs atropine). (Actual luminal HCO₃⁻ concentration in the control group was 21.4 (1.4) mmol/l, and the use of measured instead of calculated (20 mmol/l) data only marginally changed the results and did not influence the significance values.)
Intestinal bicarbonate absorption

Aspiration sites. Luminal PCO₂ did not differ from control values at the same aspiration site. We also tried to measure the secretory HCO₃⁻ flux in proximal and mid duodenum in humans and saw a 90% reduction from the bulb to the level of our mixing segment, which had a HCO₃⁻ concentration at a constant PCO₂, µmol/min per 10 cm may account for the apparent leftward displacement of the atropine curve (fig 3). Inhibition of a secretory flux with a relatively high HCO₃⁻ concentration may also account for the reduction in HCO₃⁻ concentration at the proximal aspiration site in the atropine group. The same mechanism would also reduce HCO₃⁻ concentration at the distal end of the test segment which, at a constant PCO₂, would lead to an increase in luminal acidity. Inhibition of a secretory HCO₃⁻ flux is therefore an altogether possible explanation for our findings.

Atropine had three major effects on duodenal HCO₃⁻ transport: it reduced luminal HCO₃⁻ concentration at the proximal aspiration site, it displaced the relation between HCO₃⁻ concentration and absorption to the left, and it induced significant acidification of the luminal contents at the distal aspiration site. Net absorption of a compound is the difference between the absorptive and secretory flux, and stimulation of net absorption may therefore be due to stimulation of the absorptive flux, inhibition of the secretory flux, or a combination of the two.

Atropine inhibits the secretory HCO₃⁻ flux in both the human proximal duodenum¹⁹ and rat jejunum,²⁰ and this mechanism may therefore account for our findings. Theoretically, inhibition of a (normally reabsorbed) secretory HCO₃⁻ flux with a magnitude of about 20 µmol/min per 10 cm may account for the apparent leftward displacement of the atropine curve (fig 3). Inhibition of a secretory flux with a relatively high HCO₃⁻ concentration may also account for the reduction in HCO₃⁻ concentration at the proximal aspiration site in the atropine group. The same mechanism would also reduce HCO₃⁻ concentration at the distal end of the test segment which, at a constant PCO₂, would lead to an increase in luminal acidity. Inhibition of a secretory HCO₃⁻ flux is therefore an altogether possible explanation for our findings.

There are, however, some observations that are difficult to reconcile with this interpretation. First, the secretory HCO₃⁻ flux may be very small at the level of our test segment—that is, even total inhibition of this flux may not suffice to account for the displacement of the saturation curve in the atropine experiments. Isenberg et al²¹ have directly compared the secretory HCO₃⁻ flux in proximal and mid duodenum in humans and saw a 90% reduction from the bulb to the level of our mixing segment, which had a HCO₃⁻ secretion rate of only 3–4 µmol/min per 10 cm. It is always difficult to interpret segmental differences between species, but data from rat jejunum suggest that the basal secretory HCO₃⁻ flux is even lower more distally.²² In the present study, we also tried to measure the secretory HCO₃⁻ flux by saline perfusion. HCO₃⁻ influx into the mixing segment was 11.2 (6.0) µmol/min, which generated a luminal HCO₃⁻ concentration of 1.6 (0.8) mmol/l. However, despite this very low HCO₃⁻ concentration, net HCO₃⁻ transport in the test segment was not significantly different from zero (fig 2). If a secretory flux of any quantitative magnitude had occurred at this level, it must consequently have been totally reabsorbed.

In a saturated system, a reduced intrinsic substrate load will increase uptake of an extrinsic marker—that is, inhibition of a substantial HCO₃⁻ secretion by the mucosa itself will be expected to enhance HCO₃⁻ uptake from the lumen. However, the system was not saturated, as seen from the strong concentration dependence of HCO₃⁻ absorption. It is therefore very difficult to envisage how inhibition of a minor secretory flux can account for the leftward displacement of the HCO₃⁻ absorption curve.

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To analyse further the mechanism behind the apparent stimulatory effect of atropine on HCO₃⁻ absorption, we also calculated luminal H⁺ ion concentration and luminal PCO₂ values at the proximal and distal aspiration sites. Figure 5 summarises the results. At the proximal aspiration site, there was no significant difference in luminal H⁺ ion concentration between the three groups (Kruskal-Wallis test, p = 0.09) (fig 5A). Between proximal and distal aspiration sites, H⁺ concentration increased significantly in the atropine group (p=0.01) but no significant change occurred in the control group (p = 0.09) or in the villus atrophy group (p = 0.48). Consequently, at the distal aspiration site, luminal H⁺ concentration in the atropine group was significantly higher and in the villus atrophy group significantly lower than in the control group (p<0.01).

Figure 5B summarises in a similar fashion luminal PCO₂ values at the proximal and distal aspiration sites. Luminal PCO₂ did not differ between the groups, either at the proximal or distal aspiration sites, and no significant change in PCO₂ between proximal and distal aspiration sites occurred in any of the three groups.

Discussion

Atropine had three major effects on duodenal HCO₃⁻ transport: it reduced luminal HCO₃⁻ concentration at the proximal aspiration site, it displaced the relation between HCO₃⁻ concentration and absorption to the left, and it induced significant acidification of the luminal contents at the distal aspiration site. Net absorption of a compound is the difference between the absorptive and secretory flux, and stimulation of net absorption may therefore be due to stimulation of the absorptive flux, inhibition of the secretory flux, or a combination of the two.

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jejenum, Turnberg et al obtained only partial saturation with luminal concentrations as high as 200 mmol/l. The linear part of their saturation curve was virtually identical with our own, strongly suggesting that they were studying the same transport mechanism. An important question is whether this saturation curve fully accounts for the HCO\textsuperscript{−} concentration decrease between the infusion site and the proximal site, or if a secretory HCO\textsuperscript{−} flux has to be included to account for the observed concentration changes. To test this, we used the intercept of the regression line with the HCO\textsuperscript{−} concentration of our perfusate (25 mmol/l) to estimate HCO\textsuperscript{−} absorption in the mixing segment. The expected mean absorption rate (fig 2) will be 47/2 = 23.5 µmol/min. Mean flow rate through the mixing segment (including the pressure infusion) was 6.26 (0.19) ml/min and there was no significant net water flux (0.00 (0.15) ml/min). One consequently expects a mean luminal HCO\textsuperscript{−} concentration at the proximal aspiration site of 25 − (23.5/6.26) = 21.2 mmol/l, which agrees almost exactly with the measured mean value (21.4 (1.4) mmol/l, table 1). The most likely explanation for the relatively pronounced scatter around this mean value is interindividual differences in the degree of stirring motor activity in the mixing segment, which probably increases HCO\textsuperscript{−} uptake by improving the contact with the absorbing epithelium. At least as judged from these rough calculations, HCO\textsuperscript{−} absorption thus seems to account fully for the HCO\textsuperscript{−} concentration changes at the proximal aspiration site.

A similar mode of calculation can also be used to estimate HCO\textsuperscript{−} inflow into the mixing segment. At the proximal aspiration site, the mean HCO\textsuperscript{−} “flow rate” (volume flow × [HCO\textsubscript{3}−]) was 134 µmol/min. If the mixing segment had a similar absorption rate to the test segment—that is, 20–25 µmol/min per 5 cm, one would expect an initial mean HCO\textsuperscript{−} inflow rate of 155–160 µmol/min. HCO\textsuperscript{−} was infused at a rate of about 150 µmol/min—that is, only 5–10 µmol/min remain as a possible mean value for the secretory inflow into the mixing segment. Even this value, which agrees reasonably well with both Isenberg’s data and our own NaCl experiments (our mean value was 11.2 (6.0) µmol/min), is probably an overestimation, as this mode of calculation assumes optimal stirring conditions in the mixing segment.

In the atropine group, 10 of 13 subjects followed a regression line that was displaced about 20 µmol/min per 10 cm to the left—that is, at a given concentration, HCO\textsuperscript{−} absorption was 20 µmol/min per 10 cm higher. The equation for this regression line gave an expected HCO\textsuperscript{−} absorption in the mixing segment of around 35 µmol/min which, with the mode of calculation used above, should lead to a further decrease in mean proximal HCO\textsuperscript{−} concentration value by about 3.5 mmol/l. The actually measured mean differences between controls and atropine data was 5 mmol/l (table 1). Furthermore, the three experiments in the atropine group which seemed to follow the control regression line (solid squares in fig 3) also had a proximal HCO\textsuperscript{−} concentration which was virtually identical with the mean value in the control group. Attempts to estimate the amount of HCO\textsuperscript{−} entering the mixing segment in the atropine group gave negative values (−12.9 (6.6) µmol/min)—that is, in the atro- pine group, HCO\textsuperscript{−} absorption capacity in the mixing segment was probably even higher than in the test segment.

To conclude these numeric excursions, our data are theoretically compatible with both inhibition of HCO\textsuperscript{−} secretion and stimulation of HCO\textsuperscript{−} absorption, but increased affinity of the absorptive mechanism accounts for both transport and concentration data in a strikingly consistent manner.

To obtain additional information on the transport mechanisms responsible for HCO\textsuperscript{−} absorption, we also included a small group of patients with villus atrophy in the study. The idea was that, if HCO\textsuperscript{−} absorption depends on NHE activity in the apical membrane of villus enterocytes, one would expect villus atrophy to have the opposite effect to atropine—that is, to reduce HCO\textsuperscript{−} absorption and to decrease H\textsuperscript{+} and CO\textsubscript{2} formation. As a group, the patients did exhibit significantly reduced HCO\textsuperscript{−} absorption in their test segment, but the inter-individual variation was fairly large, with “normal” absorption values occurring in some subjects. The linear correlation between HCO\textsuperscript{−} absorption and luminal HCO\textsuperscript{−} concentration was also lost in the patients, which precluded “backward” calculation of the amount of HCO\textsuperscript{−} entering the mixing segment. Another puzzling finding was that, despite their inability to absorb HCO\textsuperscript{−} in the test segment, the patients had a HCO\textsuperscript{−} concentration at the proximal aspiration site that was virtually identical with that in the controls (table 1). However, in the patients, the decrease in HCO\textsuperscript{−} concentration was almost exactly paralleled by dilution of the volume marker—that is, the mechanism was probably increased inflow of secreted fluid with a low HCO\textsuperscript{−} concentration into the mixing segment.22

The reason for the much larger variability in the HCO\textsuperscript{−} transport data in the patients is not altogether clear. Prostaglandins, which are released during inflammation, stimulate HCO\textsuperscript{−} secretion also in the distal duodenum,11 and it cannot therefore be excluded that a numerically relevant secretory HCO\textsuperscript{−} flux may have occurred in some of the villus atrophy patients. Other possibilities are interindividual differences in the degree of villus atrophy, or simply shortcomings of our fairly crude methodology. However, it should be stressed that, despite the relatively large scatter, HCO\textsuperscript{−} transport rate in the patient group was significantly different from that in the healthy subjects.

In all three groups, the same solution was infused at the same rate and one therefore also expects differences in HCO\textsuperscript{−} absorption between the groups to be reflected by changes in luminal acidity and PCO\textsubscript{2}, particularly at the distal aspiration site. Since our hypothesis was
Intestinal bicarbonate absorption

that \( \text{HCO}_3^- \) is absorbed by \( \text{Na}^+/\text{H}^+ \) exchange, which is in turn stimulated by atropine and reduced by villus atrophy; the pattern expected from the model is increased acidity and \( \text{PCO}_2 \) at the duodenaljunal junction in the atrope group, and essentially the opposite pattern in the villus atrophy group. The actual findings were slightly different: luminal acidity did show the expected profile, but luminal \( \text{PCO}_2 \) was virtually identical in all three groups.

When interpreting changes in luminal \( \text{PCO}_2 \), one has to remember that \( \text{CO}_2 \) is a very lipid-soluble gas that readily diffuses across the duodenal mucosa. The development of \( \text{PCO}_2 \) gradients therefore depends not only on production rate but also on factors affecting diffusion—that is, thickness and hydrophobicity of unstirred layers. Thus, despite a longer transport segment and a higher luminal bicarbonate concentration, Turnberg et al. had to use coupled buffer systems and a reduced perfusion rates to show a \( \text{HCO}_3^- \)-induced increase in luminal \( \text{PCO}_2 \) during triple lumen perfusion of human jejunum. In a better controlled system (perfused rat jejunum in vivo), Hubel et al. did see an increase in luminal \( \text{PCO}_2 \) with time if \( \text{HCO}_3^- \) was present in the lumen. However, the magnitude of this increase was not significantly affected by atropine.

The most likely explanation for the poor correlation between luminal \( \text{PCO}_2 \) and \( \text{HCO}_3^- \) absorption seems to be that \( \text{CO}_2 \), being a highly lipid-soluble gas, rapidly diffuses into the mucosa. The segment consequently behaves like a \( \text{PCO}_2 \)-stat system, and changes in luminal \( \text{HCO}_3^- \) concentration are primarily reflected by \( \text{pH} \) changes. Provided that one accepts the \( \text{PCO}_2 \)-stat model, the data thus suggest that atropine enhances \( \text{HCO}_3^- \) absorption by a process that involves increased proton formation from the duodenal villi.

The mechanism of action of atropine remains unknown. It seems quite clear that cholinergic neurones do participate in the control of intestinal chloride secretion but in our model, we have also postulated a cholinergic inhibitory effect on the absorbing epithelium. There is actually some evidence against this hypothesis. Both intestinal villi and crypts are supplied with muscarinic receptors, but activation of enteric neurones by electrical field stimulation does not inhibit the absorptive sodium or chloride fluxes. Another contradictory finding is that the absorptive response to a meal was not affected by luminal administration of bupivacaine, a local anaesthetic agent. One clearly has to keep an open mind about the exact mechanism behind the atrope response.

In summary, our results show that, in the interdigestive state, duodenal net \( \text{HCO}_3^- \) absorption is increased by atropine when given in a dose that blocks muscarinic receptors. A detailed analysis of the transport data suggests stimulation of active \( \text{HCO}_3^- \) absorption as the most probable mechanism. The results are compatible with interdigestive cholinergic inhibition of villus NHE activity, but the data are far too indirect to allow this conclusion.

Mechanisms for neural inhibitory control of the vectorial NHE do, however, exist and our results certainly point to the physiological role of this system. The isoform responsible for this vectorial \( \text{Na}^+ \) transport is probably NHE3, which is inhibited by high dose amiloride given from the luminal side. A good experiment would be to try to block the atrope response with high dose amiloride, preferably in a less complicated animal model.

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