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 \( \text{HCO}_3^- \) absorption, which depends on villus \( \text{Na}^+ / \text{H}^+ \) 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—\( \text{HCO}_3^- \) 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 \( \text{HCO}_3^- \) transport: it reduced \( \text{HCO}_3^- \) concentration at the proximal aspiration site, it displaced the relation between \( \text{HCO}_3^- \) concentration and \( \text{HCO}_3^- \) 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 \( \text{HCO}_3^- \) 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 \( \text{HCO}_3^- \) absorption, which depends on villus \( \text{Na}^+ / \text{H}^+ \) exchanger activity, may be tonically inhibited by an atropine sensitive cholinergic mechanism.

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Keywords: small intestine; absorption; cholinergic; muscarinic receptor; villus atrophy; coeliac disease

Several enteric neurotransmitters and gut hormones—for example, acetylcholine, 5-hydroxytryptamine, substance P, and neuropeptides—inhibit electrically silent \( \text{Na}^+ \) absorption via a \( \text{Ca}^{2+} \) dependent mechanism, but the physiological role of this system is not known. One possible function may be down regulation of epithelial transport activity in the fasting state. When the “feeding programme” is activated, ileal \( \text{Na}^+ \) absorption increases significantly, and this response is blocked by high dose amiloride, suggesting disinhibition of villus \( \text{Na}^+ / \text{H}^+ \) exchanger (NHE) activity. The absorbing enterocytes may consequently alternate between a “resting” and an active state, depending on the degree of inhibitory neural activity.

While studying the linkage between interdigestive motility and secretion in the upper small intestine in humans, we made the observation that atropine tended to enhance duodenal \( \text{HCO}_3^- \) absorption. In the proximal small intestine, bicarbonate is absorbed by \( \text{Na}^+ / \text{H}^+ \) exchanger activity—that is, with an excess of \( \text{HCO}_3^- \) in the lumen, NHE activity will be rate limiting for active \( \text{HCO}_3^- \) absorption (fig 1). If cholinergic neurones tonically inhibit NHE activity in the fasting state, one will consequently expect blockade of this inhibitory influence to enhance active \( \text{HCO}_3^- \) absorption. This effect also actually occurs when atropine is given intravenously to anaesthetised rats.

In the experiments described below, we used \( \text{HCO}_3^- \) absorption as an indirect marker for vectorial NHE activity, and tried to test the hypothesis that, in the fasting state, this activity is tonically inhibited by a cholinergic atropine sensitive mechanism. Using a modified triple...
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, and in most 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 Hydén. 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: [HCO₃⁻] = S × PCO₂ × 10^(pH−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.

CALCULATIONS OF HCO₃⁻ ABSORPTION

HCO₃⁻ absorption in the test segment was calculated from the following formula: HCO₃⁻ absorption = ([Qₚr − 0.7] × [HCO₃⁻]ₚ) − ([Qₚr − 0.7 − NFT] × [HCO₃⁻]ₚ), where Qₚr = volume flow at the proximal aspiration site (= Q × [PEG]ₚ/[PEG]₟), Q = pump rate (=6 ml/min), [PEG] = PEG concentration, 0.7 = proximal aspiration rate (ml/min), NFT = net fluid transport within the test segment (= [Qₚr − 0.7] × (1 − [PEG]ₚ/[PEG]₟)). Subscripts pr, pt, and 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
bridge amplifiers on a Grass polygraph (model 7D; Grass Instruments, Quincy, Massachusetts, USA). A contraction was defined as a phasic increase in intraluminal pressure exceeding 8 mm Hg. The frequency of contractions was calculated manually from the paper recordings every minute.

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.

EXPERIMENTAL GROUPS
The control group (n = 11) consisted of healthy volunteers, perfused with Krebs/mannitol 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/mannitol 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/mannitol and received no experimental intervention.

INTESTINAL PERFUSATES
In most experiments the intestine was perfused with a Krebs/mannitol solution with the following composition (in mmol/l): 122 NaCl, 4.7 KCl, 1.2 KH2PO4, 25 NaHCO3, 1.2 MgCl2, 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% CO2) 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/mannitol was used as perfusate in the villus atrophy group.

CALCULATIONS AND STATISTICAL EVALUATION
In an unsaturated system, HCO3− absorption will be concentration dependent. Luminal HCO3− concentration at the proximal aspiration site was significantly lower in the atropine group and therefore the concentration factor had to be corrected for when comparisons of absolute HCO3− absorption rates in the different groups were made. In the atropine group, most of the data followed a concentration-absorption line that was roughly parallel to the control line. To test if the distribution of these data differed from the control distribution, concentration values were fed into the equation for the control line, and calculated (“expected”) HCO3− absorption values were compared with actual (measured) values, under the null hypothesis that the two sets of data belong to the same population. The same calculation was performed for the control group, and the significance of the difference between the two populations was then tested with the non-parametric Mann-Whitney U test.

In the villus atrophy group, there was no consistent relationship at all between HCO3− concentration and absorption, and therefore this “mathematical” approach was considered inappropriate. However, in this group, spontaneous luminal HCO3− concentration was not significantly different from that obtained in the control group, and therefore this luminal concentration value (= 20 mmol/l) was chosen as the normalisation point for all three groups. At this concentration point, we were thus able to use actual measured data from controls and villus atrophy patients, and only had to normalise the atropine data to a concentration of 20 mmol/l, as calculated from the slope of the regression line. In this way, we were able to make a meaningful quantitative comparison of absolute HCO3− absorption rate in the three groups, at a similar luminal HCO3− concentration value.

The comparison between the three groups was performed with the non-parametric Kruskal-Wallis test and the Mann-Whitney U test. In the analysis of pH and P CO2 data, we calculated P CO2 and H+ concentration at the proximal and distal aspiration sites. The difference between the two aspiration sites, within each group, was tested by the non-parametric Wilcoxon test, and the difference between absolute values at each aspiration site between the three groups was first tested with the Kruskal-Wallis test followed, if justified, by the non-parametric Mann-Whitney U test for independent samples. p<0.05 was regarded as statistically significant.

Results
There was no significant difference in motor activity between the groups (table 1). Luminal HCO3− concentration at the entry to the test segment was not significantly different in the control and villus atrophy groups (= 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 HCO3− in the control group. During perfusion with the HCO3−-containing solution (filled triangles), there was a significant linear correlation between HCO3− absorption and HCO3− concentration measured at the proximal aspiration site (r = 0.80, p<0.01). During perfusion
Table 1  Duodenal motor activity and luminal \( \text{HCO}_3^- \) concentration at entry to test segment, in the different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Motor activity (contractions/ min)</th>
<th>( \text{HCO}_3^- ) 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 \( \text{HCO}_3^- \) absorption as a function of luminal \( \text{HCO}_3^- \) 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. \( \text{HCO}_3^- \) transport is expressed as \( \mu \text{mol/min per 10 cm} \); 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 \( \text{HCO}_3^- \) concentration and \( \text{HCO}_3^- \) absorption in the atropine group. 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 \( \text{HCO}_3^- \) absorption at a luminal \( \text{HCO}_3^- \) concentration of 20 mmol/l in the three groups. Control data and atropine data were recalculated from the slope of the regression lines in figs 2 and 3. Villus atrophy data were measured data (mean luminal \( \text{HCO}_3^- \) 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 \( \text{HCO}_3^- \)-free solution (NaCl, open triangles), \( \text{HCO}_3^- \) transport was not significantly different from zero.

Figure 3 shows individual values for \( \text{HCO}_3^- \) concentration and \( \text{HCO}_3^- \) absorption in the atropine group. In this group, there was no longer any significant correlation between \( \text{HCO}_3^- \) concentration and \( \text{HCO}_3^- \) absorption (\( r = 0.32, p = 0.28 \)). However, 10 of 13 subjects (solid squares) followed a regression line that was approximately parallel to the one obtained in the controls (which is indicated by the dashed line in fig 3). The three remaining subjects (open squares) closely followed the control regression line. To test if the displacement of the atropine data from the control regression line was statistically significant, we fitted the concentration data in the atropine group into the control regression equation, and then compared measured and expected \( \text{HCO}_3^- \) absorption rate under the null hypothesis that the atropine data were randomly distributed around the control line. This calculation showed that the atropine data were significantly displaced to the left of the control population (control distribution: 0.0 (3.6); atropine distribution: +13.8 (3.7) \( \mu \text{mol/min per 10 cm} \); p<0.05).

In the villus atrophy patients, there was no consistent relation at all between luminal \( \text{HCO}_3^- \) 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 \( \text{HCO}_3^- \) 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 \( \text{HCO}_3^- \) absorption values in all three groups at a similar luminal \( \text{HCO}_3^- \) 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 \( \text{HCO}_3^- \) absorption rate was 35.4 (3.8) \( \mu \text{mol/min per 10 cm} \), which increased to 48.0 (3.4) \( \mu \text{mol/min per 10 cm} \) after atropine administration (p<0.05 vs control data). In the villus atrophy group, the normalised \( \text{HCO}_3^- \) absorption rate was 15.2 (5.7) \( \mu \text{mol/min per 10 cm} \) (p = 0.01 vs controls, p<0.001 vs atropine). (Actual luminal \( \text{HCO}_3^- \) 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.)
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aspiration sites. Luminal P CO2 did not di
H+ ion concentration and luminal P CO2 values
luminal PCO2 values at the proximal and distal
than in the control group (p<0.01).
the villus atrophy group significantly lower
atropine group was significantly higher and in

HCO3
effects on duodenal
Atropine had three major e
V

Discussion

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 differ-
ence in luminal H+ ion concentration between
the three groups (Kruskal-Wallis test, p = 0.09)
(fig 5A). Between proximal and distal aspira-
tion sites, H+ concentration increased signifi-
cantly in the atropine group (p<0.01) but no
significant change occurred in the control
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− concen-
tration 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, inhib-
ition of the secretory flux, or a combination of
the two.

Atropine inhibits the secretory HCO3− flux
in both the human proximal duodenum20 and
rat jejunum,29 and this mechanism may there-
fore 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− concen-
tration at the proximal aspiration site in the atro-
pine group. The same mechanism would also
reduce HCO3− concentration at the distal end
of the test segment which, at a constant P CO2,
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 inter-
pretation. 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 suf-
fice 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% reduc-
tion 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 dif-
ficult to interpret segmental differences be-
tween species, but data from rat jejunum
suggest that the basal secretory HCO3− flux is
even lower more distally.20 In the present study,
we also tried to measure the secretory HCO3−
flux by saline perfusion. HCO3− inflow into the
mixing segment was 11.2 (6.0) µmol/min,
which generated a luminal HCO3− concen-
tration of 1.6 (0.8) mmol/l. However, despite this
very low HCO3− concentration, net HCO3−
transport in the test segment was not signifi-
cantly different from zero (fig 2). If a secretory
flux of any quantitative magnitude had oc-
curred at this level, it must consequently have
been totally reabsorbed.

In a saturated system, a reduced intrinsic
substrate load will increase uptake of an extrin-
sic 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 depend-
ence of HCO3− absorption. It is therefore very
difficult to envisage how inhibition of a minor
secretory flux can account for the leftward dis-
placement of the HCO3− absorption curve.

A characteristic feature of HCO3− absorption
is that it is very hard to saturate. In the
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 HCO₃⁻ concentration decrease between the infusion site and the proximal site, or if a secretory HCO₃⁻ 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 HCO₃⁻ concentration of our perfusate (25 mmol/l) to estimate HCO₃⁻ absorption in the mixing segment. The expected mean absorption rate (fig 2) will be 47.2 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 HCO₃⁻ 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 HCO₃⁻ uptake by improving the contact with the absorbing epithelium. At least as judged from these rough calculations, HCO₃⁻ absorption thus seems to account fully for the HCO₃⁻ concentration changes at the proximal aspiration site.

A similar mode of calculation can also be used to estimate HCO₃⁻ inflow into the mixing segment. At the proximal aspiration site, the mean HCO₃⁻ "flow rate" (volume flow × [HCO₃⁻]) 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 HCO₃⁻ inflow rate of 155–160 µmol/min. HCO₃⁻ 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 by about 3.5 mmol/l. The actually measured mean difference 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 HCO₃⁻ concentration which was virtually identical with the mean value in the control group. Attempts to estimate the amount of HCO₃⁻ entering the mixing segment in the atropine group gave negative values (−12.9 (6.6) µmol/min)—that is, in the atropine group, HCO₃⁻ 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 HCO₃⁻ secretion and stimulation of HCO₃⁻ 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 HCO₃⁻ absorption, we also included a small group of patients with villus atrophy in the study. The idea was that, if HCO₃⁻ 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 HCO₃⁻ absorption and to decrease CO₂ formation. As a group, the patients did exhibit significantly reduced HCO₃⁻ absorption in the test segment, but the interindividual variation was fairly large, with "normal" absorption values occurring in some subjects. The linear correlation between HCO₃⁻ absorption and luminal HCO₃⁻ concentration was also lost in the patients, which precluded “backward” calculation of the amount of HCO₃⁻ entering the mixing segment. Another puzzling finding was that, despite their inability to absorb HCO₃⁻ in the test segment, the patients had a HCO₃⁻ concentration at the proximal aspiration site that was virtually identical with that in the controls (table 1). However, in the patients, the decrease in HCO₃⁻ 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 HCO₃⁻ concentration into the mixing segment.

The reason for the much larger variability in the HCO₃⁻ transport data in the patients is not altogether clear. Prostaglandins, which are released during inflammation, stimulate HCO₃⁻ secretion also in the distal duodenum, and it cannot therefore be excluded that a numerically relevant secretory HCO₃⁻ 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, HCO₃⁻ 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 HCO₃⁻ absorption between the groups to be reflected by changes in luminal acidity and PCO₂, particularly at the distal aspiration site. Since our hypothesis was...
that HCO$_3^-$ is absorbed by Na$^+$/H$^+$ exchange, which is in turn stimulated by atropine and reduced by villus atrophy, the pattern expected from the model is increased acidity and P$\text{CO}_2$ at the duodenoejejunal junction in the atrope group, and essentially the opposite pattern in the villus atrope group. The actual findings were slightly different: luminal acidity did show the expected profile, but luminal P$\text{CO}_2$ was virtually identical in all three groups.

When interpreting changes in luminal P$\text{CO}_2$, one has to remember that CO$_2$ is a very lipid-soluble gas that readily diffuses across the duodenal mucosa. The development of P$\text{CO}_2$ 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 perfusion rate to show a HCO$_3^-$/V$\text{CO}_2$ correlation between luminal P$\text{CO}_2$ and HCO$_3^-$ during triple lumen perfusion of human jejunum. In a better controlled system (perfused rat jejunum in vivo), Hubel$^{35}$ did see an increase in luminal P$\text{CO}_2$ with time if HCO$_3^-$ was present in the lumen. However, the magnitude of this increase was not significantly affected by atropine.$^{36}$

The most likely explanation for the poor correlation between luminal P$\text{CO}_2$ and HCO$_3^-$ absorption seems to be that CO$_2$, being a highly lipid-soluble gas, rapidly diffuses into the mucosa. The segment consequently behaves like a “P$\text{CO}_2$-stat” system, and changes in luminal HCO$_3^-$ concentration are primarily reflected by pH changes. Provided that one accepts the “P$\text{CO}_2$-stat” model, the data thus suggest that atropine enhances 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$^{26}$–$^{30}$ 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 innervated by cholinergic neurones$^{31}$–$^{35}$ and are supplied with muscarinic receptors,$^{31}$–$^{35}$ but activation of enteric neurones by electrical field stimulation does not inhibit the absorptive sodium or chloride fluxes.$^3$ Another contradictory finding is that the absorptive response to a meal was not affected by luminal administration of bupivacaine, a local anaesthetic agent.$^{36}$ One clearly has to keep an open mind about the exact mechanism behind the atropine response.

In summary, our results show that, in the interdigestive state, duodenal net 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 HCO$_3^-$ absorption as the most probable mechanism. The results are compatible with interdigestive cholinergic inhibition of villus NH$^+$ activity, but the data are far too indirect to allow this conclusion.

Mechanisms for neural inhibitory control of the vectorial NH$^+$ do, however, exist$^7$ and our present results certainly give further evidence for the physiological role of this system. The isoform responsible for this vectorial NH$^+$ transport is probably NH$^+$E3, which is inhibited by high dose amiloride given from the luminal side.$^{37}$ A good experiment would be to try to block the atropine response with high dose amiloride, preferably in a less complicated animal model.

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