Acid stimulated alkaline secretion in the rabbit duodenum is passive and correlates with mucosal damage

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SUMMARY Low luminal acid concentrations stimulate alkaline secretion (AS) by the duodenal mucosa. We investigated acid stimulated alkaline secretion by proximal rabbit duodenal mucosa in an Ussing-chamber under different luminal acid concentrations and its relation to mucosal damage. Luminal alkalinisation and potential difference (PD) were measured and mucosal damage was investigated histologically. Luminal acid caused an increase of alkaline secretion over baseline ($0.95 \pm 0.19 \mu\text{Eq}/\text{cm}^2/10 \text{ min}$; $n=55$): 0.1 mmol/l : 7%, 1 mmol/l : 17%, 5 mmol/l : 22%, 10 mmol/l : 33%, 20 mmol/l : 34%, 50 mmol/l : 39%, 100 mmol/l : 27%. At acid concentrations of 10 mmol/l and above the PD fell from $2.0 \pm 1.0 \text{ mV}$ to zero. Histology showed $[\text{H}^+]$ -dependent mucosal damage ranging from villus tip lesions to deep mucosal injury. Stimulation of alkaline secretion was not specific for acid. Ethanol (14%) stimulated alkaline secretion by 26%, and 28% ethanol by 40% over baseline. Ouabain and/or anoxia sensitive (active) alkaline secretion constituted 80% and 100% respectively of basal alkaline secretion. After exposure to various luminal acid concentrations passive diffusion (sensitive only to removal of nutrient HCO_3^-) was solely responsible for the rise in alkaline secretion. Only after 14% ethanol a small rise in ouabain and/or anoxia sensitive HCO_3^- transport was observed. Under the conditions of this study stimulation of duodenal alkaline secretion is not specific for luminal acid, but occurs also with luminal ethanol; both agents stimulate alkaline secretion depending on their concentration. In this model passive diffusion of HCO_3^- associated with increasing mucosal damage is the major component of the rise in alkaline secretion.

The mechanisms which enable the duodenal mucosa to resist luminal acid are not fully understood. Several studies have shown that the duodenal mucosa transports bicarbonate into the lumen.^{1–3} It has been proposed that this is important for disposal of luminal acid and thus for mucosal protection.⁴ The alkaline secretion can be stimulated by low concentrations of luminal acid. This has been shown in various species *in vivo* and *in vitro*, and it was presumed to be a physiological mechanism.^{5–12}

The aim of the present study was to investigate the

influence of different acid loads on HCO_3^- transport by the duodenal mucosa *in vitro* to gain further insight about the role of active and passive transport in acid stimulated alkaline secretion and the association of alkaline secretion with mucosal damage.

Methods

ANIMALS

A segment of the proximal duodenum (approximately 3 cm from the pylorus) of anaesthetised female New Zealand white rabbits (mean weight: 3 kg, Ivanovas, Kissleg, FRG) was excised. The external muscle layer was separated immediately by blunt dissection in an unbuffered, aerated solution at

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Received for publication 24 August 1987.

room temperature and mounted as a membrane horizontally between the two halves of an *in vitro* chamber as originally described by Sjöstrand.¹³ The area of the exposed mucosal surface was 2 cm². The volume of the chamber was 6 ml. The nutrient solution was buffered to pH 7.4 with 25 mmol/l HCO₃⁻ and contained in mmol/l: 122 NaCl, 5 KCl, 2 CaCl₂, 1.3 MgSO₄, and 20 glucose. This solution was gassed with 95% O₂/5% CO₂. No buffers were used on the luminal side where a NaCl (154 mmol/l) solution was gassed with 100% O₂ (prewashed in KOH, 4 M). The temperature in the bath was maintained at 37°C by means of a water jacket. Luminal alkalization was measured at pH=7.4. The luminal pH was kept constant by infusion of 100 mmol/l HCl under automatic control from a pH-stat-system (Radiometer, Copenhagen, Denmark). After recording of steady state alkaline secretion for 30 minutes, the luminal solution was changed to one containing different concentrations of HCl with NaCl added to achieve a total ionic strength of 308 mOsmol/l. In experiments designed to examine the effects of ethanol, the luminal solution was changed to one containing 14% or 28% ethanol in addition to NaCl, 154 mmol/l. After 10 minutes of exposure the acid or ethanol containing solution was washed out by 15–20 changes of the luminal bathing fluid with NaCl, 154 mmol/l. Measurement of alkaline secre-

tion was restarted when the luminal pH again reached pH=7.4. The time required to reach this pH spontaneously was 10–20 minutes after exposure to 1 mM HCl, but 60–80 minutes were required after 10 mmol/l HCl and 100–140 minutes after 50 mmol/l HCl. After reappearance of luminal alkalisation at pH=7.4 and irrespective of the time interval to the injury, measurable alkaline secretion rose quickly within 30–40 minutes to reach a steady state that was maintained for the remainder of the experiment. Therefore the means of the 3 highest consecutive steady state values of alkaline secretion were used for subsequent statistical analysis and presentation of the data. The PD was measured every 10 minutes between 2 agar bridges (KCl, 3 M) connected through two calomel reference electrodes (K 4040, Radiometer, Copenhagen, Denmark) to a voltmeter (pHM 82, Radiometer).

In order to characterise components of active transport and to differentiate between active and passive transport of HCO₃⁻, ouabain (Sigma Chemie GmbH, Munich, FRG) was added in some experiments to the nutrient solution to a final concentration of 10⁻³ M, and/or all metabolism-dependent transport was inhibited by anoxia (gassing change to 95% N₂/5% CO₂ on the nutrient side and 100% N₂ on the luminal side). To inhibit the passive component of bicarbonate secretion after ouabain and/or anoxia treatment the nutrient solution was changed for a bicarbonate free one. In this solution HCO₃⁻ was replaced by H₂PO₄⁻ or TRIS and gassed with 100% O₂. At the end of each experiment the tissue was carefully removed from the chamber, pinned to a corkplate and fixed with 5% formalin at pH=7. Three sections taken 3 mm apart were then embedded in paraffin. From these sections a total of at least six (two of each block) semithin (2 µm) sections were cut and stained with haematoxylin and eosin for light microscopy. The morphological evaluation was carried out by an unbiased observer, who was unaware of the experimental conditions.

Mucosal damage was evaluated both qualitatively and quantitatively. In order to estimate the depth of damage we used the following scoring system: stage 0: no damage visible in light microscopy; stage 1: villus tip lesion; stage 2: damage confined to the upper half of the villi; stage 3: 50 up to 80% of villus height affected by necrosis; stage 4: lesion as far as the base of the crypt; stage 5: full thickness necrosis.

The extent of epithelial damage was assessed by counting all villi from the six different sections and by calculating the percentage of damaged villi for each duodenal sheet. The 'damage index' was calculated using the formula:

$$\text{DI (damage index)} = \text{stage (0–5)} \times \text{extent (\%)} \quad (1)$$

Fig. 1 Alkaline secretion (AS) and potential difference (PD) after exposure to different concentrations of acid (control: $n=8$, 0.1 mmol: $n=4$, 1 mmol: $n=6$, 5 mmol: $n=6$, 10 mmol: $n=6$, 20 mmol: $n=7$, 50 mmol: $n=8$, 100 mmol: $n=10$) and ethanol (14%: $n=6$, 28%: $n=6$). Values are means (SE).

Fig. 2 (a) Lesion confined to the upper part of the villus (stage 2) caused by 5 mmol HCl; note: propria tissue (*) remains intact. BG: Brunner's glands. mm: muscularis mucosae. ns: nutrient side. Demarcation border marked by → ← (H & E). (b) 50% of villus height damaged after 10 mmol HCl (stage 3); note: necrotic propria tissue marked by *. Demarcation border marked by → ← (H & E). (c) 80% of villus height affected by necrosis after 20 mmol HCl (stage 3); note: the crypt cells remain intact (*). Demarcation border marked by → ← (H & E). (d) Lesion as far as the base of the crypts (stage 4) caused by 50 mmol HCl. Necrotic crypt cells marked by * (H & E).

STATISTICAL ANALYSIS

Statistical analysis of the data was performed using the Mann-Whitney test and Kendall's Tau correlation coefficient for unpaired data. All values are given as means (SE).

Results

Under basal conditions the mucosa maintained a stable PD and secretory rate for at least six hours. Mean values of PD and alkaline secretion for 76

tissues were 1.96 (0.41) mV and 0.95 (0.19) $\mu\text{Eq}/\text{cm}^2$ 10 min.

EFFECT OF LUMINAL ACID

In the first series of experiments the influence of different luminal acid concentrations on duodenal HCO_3^- transport and PD were investigated ($n=55$). Luminal acid exposure was followed by an increase of alkaline secretion over baseline that was dependent in magnitude on the luminal acid concentration (Fig. 1). Higher concentrations of acid produced a

Fig. 2c

Fig. 2d

stronger stimulation of alkaline secretion than low concentrations. Above 10 mmol/l HCl there was no further stimulation of alkaline secretion. The PD, which remained stable during and after exposure to 0.1 mmol/l, 1 mmol/l, and 5 mmol/l HCl was reduced after treatment with 10 mmol/l HCl and higher acid concentrations. The suggestion, that this fall in PD indicated mucosal damage was supported by the results of the histological evaluation of the mucosae. Treatment with 0.1 mmol/l HCl caused no visible damage. The exposure to 1 mmol/l HCl induced lesions of apical mucosal cells and occasionally tip necrosis of villi. 5 mmol/l HCl caused mucosal damage confined to the upper parts of the villi, the propria tissue in the villus axis remained intact (Fig. 2a). In contrast to this, mucosal damage caused by 10 mmol/l HCl affected at least 50% of villus height in

83.7 (2.7%) of all villi (Fig. 2b). Up to 80% of villus height were necrotic after luminal exposure to 20 mmol/l HCl (Fig. 2c). Large defects in the epithelial continuity were noticeable and the stroma of the villi was exposed. Damage after treatment with 50 mmol/l HCl was even more extensive with necrotic changes reaching down to the crypt basis (Fig. 2d). One hundred millimoles HCl caused full thickness necrosis of the tissue.

EFFECT OF ETHANOL

In order to investigate whether these stimulating properties on duodenal alkaline secretion were specific for luminal HCl, a series of mucosae was exposed to ethanol. After treatment with 14% ethanol the alkaline secretion increased by 25.3% above baseline with no change in PD, 28% ethanol

produced an increase of 45.1% and the PD fell to zero. Histologic evaluation revealed no visible damage after 14% ethanol treatment, but severe damage after 28% luminal ethanol comparable with that seen after 10 mmol/l HCl.

EFFECT OF OUABAIN AND ANOXIA

Ouabain (10^{-3} M) on the nutrient side inhibited basal alkaline secretion by 80% within 90 minutes, concurrent with a reduction of PD to zero, indicating that most of the duodenal alkaline secretion is Na^+ -dependent and active. The remaining alkaline secretion after ouabain treatment could be completely abolished by additional removal of O_2 , indicating that passive (paracellular) diffusion of HCO_3^- from the serosal solution does not contribute to basal alkaline secretion in the rabbit isolated duodenum. After exposure to the various concentrations of acid or ethanol as indicated in Figure 3, the relative (%) inhibition of stimulated alkaline secretion by ouabain decreased, but the absolute amount of inhibition

Fig. 3 Effect of ouabain and anoxia on acid-stimulated alkaline secretion. Ouabain data represent values after 90 minutes of treatment. Anoxia data are values at 15 minutes after change of gassing. Values are means (SE), $n=6$ each group.

remained the same (not significantly different from the amount of inhibition under baseline conditions). Similarly there was no significant change in the absolute amount of alkaline secretion-inhibition achieved by anoxia after stimulation with various concentrations of luminal HCl. Anoxia sensitive alkaline secretion was, however, slightly increased after exposure to 14% ethanol by 21.7% and decreased by 9.9% after 28% ethanol. The remaining alkaline secretion after treatment with ouabain ($n=3$) and/or anoxia ($n=6$) could only be abolished by removal of HCO_3^- from the nutrient solution (Fig. 4). Thus most of the increase of alkaline secretion at least after acid exposure seems to be passive and insensitive to either ouabain or anoxia. Ethanol-stimulated alkaline secretion was sensitive to anoxia after 14%, but not after 28% ethanol.

MORPHOMETRY

Morphometry showed a direct correlation between the luminal acid concentration and the morphologic pattern of tissue injury in respect to the extent as well as to the depth of mucosal damage (Table). When the damage index was plotted against the per cent increase of alkaline secretion after exposure to luminal acid (0.1–50 mmol/l) a significant linear correlation was found between the rise in alkaline secretion and the severity of mucosal damage (Fig. 5).

Discussion

Simson *et al.*² suggested that alkaline secretion in the amphibian duodenum *in vitro* consists to 50–60% of active transcellular transport of HCO_3^- , 30–40% passive diffusion down a concentration gradient and less than 10% by HCO_3^- from endogenous sources within the epithelium. Although comparable *in vitro* studies of mammalian duodenal mucosa are not available our results indicate, that passive shunt conductance of HCO_3^- (serosa to mucosa) in the rabbit duodenum under control conditions is negligible. An equally low paracellular permeability has previously been found in lower parts of the rabbit small intestine – that is, ileum, where shunt conductance for HCO_3^- was shown to be less than 10%.¹⁴ As these, and our studies determined net luminal alkaline secretion, however, we cannot exclude that secretion of H^+ or organic acids such as lactate or mucosal CO_2 fluxes may have masked a small amount of passive HCO_3^- secretion under the conditions of our experiments.

The stimulation of alkaline secretion by acid in the stomach and duodenum was first described by Heylings in the frog.^{5,6} This was later shown by others in the rat,¹⁰ dog,⁹ cat,⁷ rabbit¹², and in man.¹¹ This

Fig. 4 Effect of removal of bicarbonate from the nutrient side on persisting alkaline secretion following anoxia. Values are means (SE), $n=6$.

implies that this is a physiological mechanism mediated by prostaglandins enabling the duodenal mucosa to withstand luminal acid. Our experiments have clearly shown that in the rabbit the rise of alkaline secretion in response to luminal acid correlates with the mucosal damage produced by HCl. It should be noted, however, that the rabbit is a continuous feeder and basal alkaline secretion *in vivo* is rather high, probably because of a continuous endogenous drive by prostaglandins as suggested by Flemström and Garner.¹ This high basal alkaline secretion is refractory to further stimulation by exogenous prostaglandins. Therefore this particular

Table Mucosal damage after exposure to luminal acid

mmol HCl	n	% damaged villi	Stage	Damage index (for 10 min)
0.1	6	0	0	0
1	6	5.93 (1.04)	1	5.93
5	6	83.67 (2.7)	2	167.34
10	6	85.83 (2.05)	3	257.49
20	7	95.97 (0.38)	3	287.91
50	8	97.42 (0.34)	4	389.68
100	10	100	5	500.0

Fig. 5 Correlation of alkaline secretion (AS given as per cent increase above baseline levels) and mucosal damage (given as damage index) after luminal exposure to different concentrations of HCl: 0.1 mmol ($n=4$), 1 mmol ($n=6$), 5 mmol ($n=6$), 10 mmol ($n=6$), 20 mmol ($n=7$), 50 mmol ($n=8$) for 10 minutes. $y=111.4x+0.074$ ($p<0.001$, Kendall's Tau correlation coefficient).

mechanism to respond to luminal acid may not be operative in our preparation.

It was also shown that the stimulation of alkaline secretion is not specific for acid and occurs also after luminal ethanol exposure. As ethanol was a very hypertonic solution we can assume that its effect as a damaging agent and stimulant of alkaline secretion was unspecific and might be produced by other hypertonic solutions, too. Increased alkaline secretion after mucosal damage was described previously by Svanes¹⁵ who treated bullfrog gastric mucosa with NaCl, 1 M. Shortly after damage luminal alkalisation occurred, much higher than usually measured in this tissue. Further experiments revealed that this was mainly passive diffusion of HCO_3^- . Acid stimulated alkaline secretion in the rabbit duodenum is also a passive phenomenon. The amount of inhibition induced by ouabain or anoxia was unchanged after any of the acid concentrations used in this study. Therefore most of the increase in alkaline secretion after acid exposure occurred by passive diffusion that was only abolished by removal of HCO_3^- from the nutrient bath. An increase in passive permeability in response to luminal acid exposure was recently reported by Wilkes and Garner⁸ in experiments on the rat using 10 mmol/l acid. The increase of mucosal permeability in these experiments was demonstrated with markers such as inulin and radiolabelled urea. This increase was not seen after topical application of prostaglandin E_2 . Although most of the authors reporting acid stimulated alkaline secretion in the duodenum did not carry out histology on their tissues to exclude mucosal damage, there seem to exist two responses to luminal acid: either an active prostaglandin mediated HCO_3^- transport for protection of the intact mucosa, or a passive transmucosal flow of HCO_3^- protecting the repair process in the duodenum. These are necessary mechanisms and their predominance is dependent on the acid tolerance of the duodenal mucosa in different species.

Part of this work have previously been published in abstract form (*Gastroenterology* 1986; **90**: 1678).

References

- 1 Flemström G, Garner A, Nylander O, Hurst BC, Heylings JR. Surface epithelial HCO_3^- transport by mammalian duodenum in vivo. *Am J Physiol* 1982; **243**: G348–58.
- 2 Simson JNL, Merhav A, Silen W. Alkaline secretion by amphibian duodenum. I. General characteristics. *Am J Physiol* 1981; **240**: G401–8.
- 3 Flemström G, Heylings JR, Garner A. Gastric and duodenal HCO_3^- transport in vitro: effects of hormones and local transmitters. *Am J Physiol* 1982; **242**: G100–10.
- 4 Flemström G, Garner A. Gastroduodenal HCO_3^- transport: characteristics and proposed role in acidity regulation and mucosal protection. *Am J Physiol* 1982; **242**: G183–93.
- 5 Heylings JR, Garner A. Influence of luminal acidification on bicarbonate transport by gastric and duodenal isolated mucosae. *Prostaglandins* 1981; **21**: suppl: 67–71.
- 6 Heylings JR, Garner A, Flemström G. Regulation of gastroduodenal HCO_3^- transport by luminal acid in the frog in vitro. *Am J Physiol* 1984; **246**: G235–42.
- 7 Garner A, Hurst BC, Heylings JR, Flemström G. Role of gastroduodenal HCO_3^- transport in acid disposal and mucosal protection. In: Case RM, Garner A, Turnberg LA, Young JA, eds. *Electrolyte and water transport across gastrointestinal epithelia*. New York: Raven Press, 1982: 235–52.
- 8 Wilkes JM, Garner A, Peters TJ. Duodenal epithelial permeability in relation to stimulation of alkaline secretion [Abstract]. *Gut* 1986; **27**: A604.
- 9 Konturek SJ, Bilski J, Tasler J, Laskiewicz J. Gastro-duodenal alkaline response to acid and taurochlorate in conscious dogs. *Am J Physiol* 1984; **247**: G149–54.
- 10 Isenberg JJ, Smedfors B, Johansson C. Effect of graded doses of intraluminal H^+ , prostaglandin E_2 and inhibition of endogenous prostaglandin synthesis on proximal duodenal bicarbonate secretion in unanaesthetized rats. *Gastroenterology* 1984; **88**: 303–7.
- 11 Isenberg JJ, Hogan DL, Koss MA, Selling JA. Human duodenal mucosal bicarbonate secretion. Evidence for basal secretion and stimulation by hydrochloric acid and a synthetic prostaglandin E_1 analogue. *Gastroenterology* 1986; **91**: 370–8.
- 12 Wenzl E, Feil W, Starlinger M, Schiessel R. Alkaline secretion: a protective mechanism against acid injury in rabbit duodenum. *Gastroenterology* 1987; **92**: 709–15.
- 13 Sjöstrand SE, Rydberg B, Olbe L. Analysis of the action of cimetidine and metiamide on gastric acid secretion in the isolated guinea pig mucosa. *Naunyn Schmiedeberg's Arch Pharmacol* 1977; **296**: 139–42.
- 14 Smith PL, Cascairo MA, Sullivan SK. Sodium dependence of luminal alkalization by rabbit ileal mucosa. *Am J Physiol* 1985; **249**: G358–68.
- 15 Svanes K, Ito S, Takeuchi K, Silen W. Restitution of the surface epithelium of the in vitro frog gastric mucosa after damage with hyperosmolar sodium chloride. Morphologic and physiologic characteristics. *Gastroenterology* 1982; **82**: 1409–26.

1 Flemström G, Garner A, Nylander O, Hurst BC, Heylings JR. Surface epithelial HCO_3^- transport by