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Cl^- Dependence of HCO_3^- Transport in Frog Gastric Mucosa

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ABSTRACT

Frog (Rana temporaria) fundic mucosae in vitro were pretreated with the histamine H_2 receptor antagonist Metiamide (10^{-3}M, nutrient side) until net H^+ secretion had ceased and a steady rate of HCO_3^- transport (luminal alkalinization) was titrated. Removal of Cl^- with SO_4^{2-} or isethionate replacement from solutions bathing both sides of the mucosa abolished luminal alkalinization. Readdition of Cl^- to the luminal side only reestablished full rates of HCO_3^- transport. Nutrient (serosal) side Cl^- had no effect in this aspect. The results support the previous suggestion that the gastric HCO_3^- transport process is located at the luminal membrane of the surface epithelial cells and indicate that it occurs by (electroneutral) HCO_3^-/Cl^- exchange.

INTRODUCTION

Studies on amphibian (3,7) gastric mucosa in vitro and on guinea-pig (12), dog (2, 14, 19) and rabbit (25) stomachs have demonstrated that gastric mucosa, in addition to secreting H^+ ions also transport HCO_3^- into the gastric lumen. The prevailing net acidity of gastric secretion is thus the difference between a larger transport of H^+ ions and a smaller transport of HCO_3^-. The HCO_3^- transport is very probably a property of the surface epithelial cells in both fundus and antrum (3). It depends on tissue metabolism and is stimulated by cholinergic agents, dibutyryl cyclic GMP (3), ulcero-protective prostaglandins (13) and the hormones glucogon and cholecystokinin (Flemström et al., unpublished results). It is inhibited by several potential ulcerogens and insensitive to well known stimulants of gastric H^+ secretion such as dibutyryl cyclic AMP, histamine or gastrin. Properties of gastric HCO_3^- transport and methods used for its determination are described in detail in recent reviews (1, 6, 17).

Unstimulated (basal) HCO_3^- transport amounts, depending on the species, to only 2 - 10% of maximal H^+ transport and would offer no useful protection against acid if neutralization of H^+ by HCO_3^- took place in the gastric intraluminal bulk solution. It has therefore been proposed that the active transport

of HCO_3^- protects the mucosa by alkalinization of the immediate vicinity of the luminal cell membranes of the epithelium (3, 4). The visco-elastic mucous gel adherent to the mucosa has properties suited to maintain HCO_3^- at this surface (1).

The aim of the present study was to gain information about Cl^- dependence of gastric transport of HCO_3^-. Experiments were performed with isolated mucosae which enables changes of the ion environment on both sides of the epithelium.

METHODS

Frogs (Rana temporaria) obtained from Firma Panzer, Lauingen-Donau, FRG were kept in tap water at 10°C for up to two months and forcefed with liver (~200 mg) once a week. After killing of the animals by decapitation, the stomach was removed and the mucosa separated from the rest of the stomach wall by blunt dissection in an unbuffered, preoxygenated solution. The mucosa (exposed area 1.8 cm^2) was then mounted as a membrane between the two halves of an in vitro chamber (3). Each side of the mucosa was bathed with 20 ml of solution circulated by a gas lift. The luminal (secretory) side solution was always unbuffered and gassed with 100% O_2, prewashed in $Ba(OH)_2$ to avoid possible traces of CO_2. The pH on this side was kept constant at pH 7.40 by infusion of solutions containing 5 mM HCl or (in experiments with sulphate or isethionate solutions) 2.5 mM H_2SO_4, under automatic control from a pH-stat system (Titrator TTT2 and Autoburette ABU 13, Radiometer, Copenhagen). The nutrient (serosal) side solution was buffered with HCO_3^- (17.8 mM) and phosphate (0.8 mM) and gassed with gas mixture of 95% O_2 and 5% CO_2. The standard luminal and nutrient side solutions contained (in mM): Na^+ 102.4, K^+ 4.0, Ca^{2+} 1.8, Mg^{2+} 0.8 and Cl^- 91.6, mannitol and SO_4^{2-} being added to the luminal side to achieve iso-osmolarity (calculated osmolarity 221.8 mosM). The transepithelial electrical potential difference was measured via two matched calomel electrodes and recorded on a high-input impedance voltmeter. Electrical resistance was determined from the immediate change (~1 sec) change in the potential difference after sending current (30 $\mu A/cm^2$) through the mucosa in either direction. Experiments were performed at 20°C.

The mucosae were always pretreated with the histamine H_2 receptor antagonist Metiamide (10^{-3}M, nutrient side) until net H^+ secretion had ceased and a steady rate of luminal alkalinization been recorded for 45 min or longer. This drug was then present throughout the experiments. After a control period, Cl^- was removed by change of the standard secretory and nutrient solutions for solutions in which all Cl^- (91.6 mM) had been replaced by SO_4^{2-} (mannitol to iso-osmolarity) or isethionate (2-hydroxy-ethyl-sulphonate). To ensure removal of Cl^-, the mucosae were always washed repeatedly during a 15 min period with these solutions.

RESULTS

Removal of Cl^- from both sides of the mucosa decreased luminal alkalinization to values too low to be titrated at a luminal side pH of 7.40. Readding Cl^- to only the luminal side reestablished normal (control) rates of alkalinization. Nutrient side Cl^- was without effect in this respect (Table 1). Immediately after change to Cl^- free solutions, the pH on the luminal side was 7.00. A rise in the luminal pH above this value was used as a further test for residual HCO_3^- transport. With isethionate on both sides on the mucosa there was practically no increase in pH (<0.02 pH-units/30 min). With SO_4^{2-} on both sides, or with Cl^- on the nutrient and SO_4^{2-} on the luminal side, the luminal pH increased somewhat (0.10 ± 0.02 pH-units/30 min, means±SE, n = 12). This increase was much smaller than that observed with Cl^- on both sides (0.44 ± 0.07, n = 7).

Table 1. Effects of replacement of Cl^- on HCO_3^- transport and electrical properties of frog gastric mucosa.

Luminal/nutrient	PD (mV)	Resistance (ohm ·cm^2)	HCO_3^- (μmoles, h^{-1},cm^{-2})
Cl^-/Cl^-	18.3 ± 1.3	348 ± 35	0.33 ± 0.04
SO_4^{2-}/SO_4^{2-}	13.4 ± 0.8	497 ± 50	0
Cl^-/SO_4^{2-}	0.9 ± 0.8	433 ± 57	0.28 ± 0.05
Cl^-/Cl^-	22.2 ± 1.6	406 ± 82	0.43 ± 0.13
SO_4^{2-}/SO_4^{2-}	20.3 ± 1.7	579 ± 98	0
SO_4^{2-}/Cl^-	29.7 ± 1.8	392 ± 75	0
Cl^-/Cl^-	22.3 ± 1.1	438 ± 53	0.27 ± 0.05
Iseth.$^-$/Iseth.$^-$	13.4 ± 1.4	690 ± 71	0
Cl^-/Iseth.$^-$	-0.3 ± 1.5	530 ± 64	0.34 ± 0.08

HCO_3^- transport in fundic mucosa was unmasked by inhibition of H^+ secretion with the histamine H_2 receptor antagonist Metiamide (10^{-3}M, nutrient side). The bathing solutions were changed as indicated to solutions containing SO_4^{2-} or isethionate$^-$ instead of Cl^-. Mean values ± SE of transepithelial electrical potential difference (PD), electrical resistance and rate of luminal alkalinization (HCO_3^-) during the least 30 min of three consequtive 90 min periods are presented. With each type of ion replacement, n = 6.

Removal of Cl^- from solutions bathing both sides of the mucosa decresed the transepithelial elecetrical potential difference and increased the electrical

resistance. The latter effect was greater in experiments where Cl^- had been replaced with isethionate than if SO_4^{2-} was used.

Readdition of Cl^- to either side of the mucosa decreased the electrical resistance. The potential difference (lumen negative) was greater than in controls if Cl^- was present on the nutrient side only and smaller than in controls if Cl^- was present on the luminal side only. All changes were statistically significant ($p < 0.05$ or less, students t-test).

DISCUSSION

Teorell investigated counterdiffusion of acid and base in a membrane model as early as in 1936 and as pointed out by him (22, 23), the concentration profiles will depend on the amounts of acid and base available for diffusion, the structure and charge of the boundary where diffusion takes place and on the presence of other ions. Based on these concepts, gastric neutralization of H^+ by HCO_3^- has been proposed to occur as illustrated in Fig. 1. H^+ ions diffuse from the gastric intraluminal bulk solution into the surface mucous gel where the acid is neutralized by HCO_3^- transported into this boundary by the surface epithelial cells.

The rate of HCO_3^- transport is increased by E and F-type prostaglandins in amphibian isolated mucosa (13), dogs (2, 14, 19) and man (16) and this probably contributes to the well known ulceroprotective action of these agents. Gastric mucus per se has been reported to retard diffusion of H^+ ions (24), suggesting that there are (positive) charged groups in this gel. Carbenoxolone prevents gastric ulceration without stimulating HCO_3^- (or inhibiting H^+)transport (Rees et al., unpublished results). Recent results with optical methods suggest that this drug acts by increasing the thickness of the mucous layer (18). Concepts and theories derived from experiments with gels and artificial membranes are thus applicable to and useful in studies of the physiological and pharmacological problem of gastric mucosal resistance to acid.

The concept of HCO_3^- transport protecting the gastric mucosa has gained support not only from studies of inhibitors and stimulants of this transport but also from the recent demonstration with microelectrode techniques of a pH gradient across the layer of mucus adherent to the gastric mucosa in rabbits (25); the pH at the cell surface being slightly alkaline (mean pH 7.59) in spite of that in theluminal bulk solution being acid (mean pH 2.36). This pH gradient as abolished by administration of inhibitors of gastric mucosal HCO_3^- transport such as acetazolamide, CN^- or aspirin.

The present experiments demonstrate that presence of Cl^- ions on the luminal side is necessary for transport of HCO_3^- into the luminal solution. Previous studies (3) have shown that the concentration of acetazolamide necessary for inhibition of gastric HCO_3^- transport in vitro is considerably smaller if the

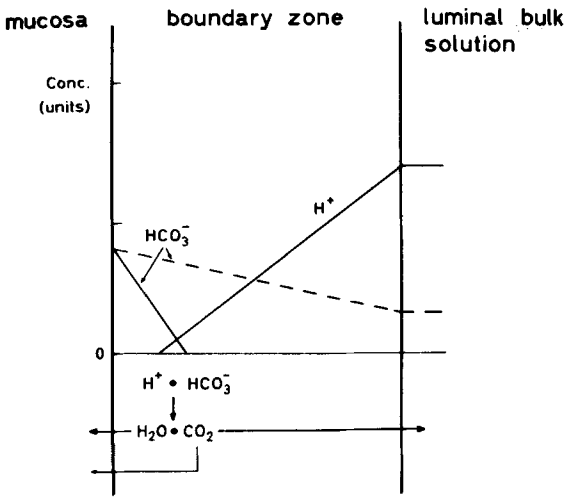

Fig. 1. Model for neutralization of H^+ by HCO_3^- in the surface mucous gel adherent to the luminal surface of gastric epithelium. Free HCO_3^- (dashed line) appears only in non-acid bulk solutions. With acid bulk solutions (solid lines), HCO_3^- is converted to CO_2. The latter may appear in the lumen or be used by the parietal cells in the H^+ secretory process.

drug is applied to the luminal (10^{-4}M) than to the nutrient (10^{-2}M) side. Carbonic anhydrase activity of the surface epithelial cells is located in the apical cytoplasmatic matrix and microvillar cores (21). The combined data strongly suggest that HCO_3^- transport occurs by a HCO_3^-/Cl^- exchange process at the luminal membrane of these cells. The process is probably electroneutral since inhibition or stimulation of HCO_3^- transport are associated with no or only very small changes in the transepithelial electrical potential difference or resistance (6). Stimulation of HCO_3^- transport in isolated mucosa by calcium ions has been reported to be associated with a rise in the transepithelial electrical potential difference (4). Further studies (9) have suggested, however, that this rise is an effect of calcium unrelated to the stimulation of HCO_3^- transport.

Removal of Cl^- from both sides of the mucosa (Table 1) decreased the transepithelial electrical potential difference and increased electrical resistance (decreased conductance). Both active transport of Cl^-, contributing to the potential difference, and passive diffusion of this ion, accounting for part of tissue conductance, have been demonstrated in gastric mucosa (11, 15, 20). The present findings are in line with these previous results. Presence of Cl^- on only the nutrient side increased, and Cl^- on only the luminal side decreased the transepithelial electrical potential difference. This very probably reflects Cl^- diffusion potentials.

Further knowledge about stimulatory and inhibitory pathways for gastric HCO_3^- transport as well as the formation, structure and charge of the surface mucous layer seems important. The present results indicate that the presence of Cl^- ions in the surface gel is important for maintenance of the HCO_3^-

transport. Finally, metabolic-dependent transport of HCO_3^- occurs also in the proximal part of duodenum in vitro (5, 8) and in vivo (10) and as in the stomach, transport is inhibited by potential ulcerogens and stimulated by prostaglandins and some hormones.

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