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Histamine Receptor Effects on Dissipation of an Intracellular Proton Gradient of Isolated Gastric Mucosal Surface Cells[1] (42557)

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Abstract. The effects of histamine and several H_1 and H_2 receptor agents on Na^+/H^+ and Cl^-/HCO_3^- exchange systems of isolated gastric mucosal surface cells were studied. The cells were acid-loaded by the NH_4Cl prepulse technique and the spontaneous Na^+- and HCO_3^--induced dissipation of the intracellular proton gradient (pH_i) was followed using the metachromatic dye acridine orange. Histamine (10^{-2-5} *M*) stimulates HCO_3^--induced dissipation of the pH_i but has no effect on Na^+-induced or spontaneous dissipation. The H_1 agonist 2-(2-aminoethyl)pyridine and the H_2 agonist dimaprit also have no effect on Na^+-induced or spontaneous pH_i dissipation. However, both of these agents mimic the effect of histamine on HCO_3^--induced dissipation, but only at a higher concentration (10^{-3} *M*). The combination of 2-(2-aminoethyl)pyridine and dimaprit produces a histamine-like effect at lower concentrations (10^{-5} and 10^{-4} *M*). The effects of histamine are blocked by either the H_1 antagonists diphenhydramine and pyrilamine or the H_2 antagonists cimetidine and SKF 93479. The results suggest that the effect of histamine on HCO_3^--induced dissipation of a pH_i in gastric mucosal surface cells is mediated through a coordinated mechanism involving both H_1 and H_2 receptor sites.

The role of histamine in the control of gastric acid secretion has been extensively studied and is generally well understood from a conceptual point of view. However, other effects of histamine in the gastric mucosa, such as its interaction with mucosal surface cells, are virtually unknown. Isolated gastric mucosal surface cells have been shown to regulate their intracellular pH (pH_i) by means of ion exchange systems involving Na^+/H^+ and Cl^-/HCO_3^- (1–3). The purpose of this study was to determine the effects of histamine and several H_1 and H_2 receptor agents on the regulation of intracellular pH by acid-loaded surface cells isolated from rabbit gastric mucosa.

Methods. New Zealand white rabbits (2–4 kg) were anesthetized using a combination of xylazine, 5 mg/kg im, ketamine, 40 mg/kg im, and pentobarbital, 1 ml iv, prior to removing the stomach. Surface cells were obtained as previously described (2, 4). Briefly, minced mucosal fragments were incubated in solution A (see below) containing protease, 0.01% (w/v), and hyaluronidase, 0.05%, for 20 min at 37°C with 100% O_2 and constant stirring. The supernatant was decanted and filtered through a 4 × 4 gauze. Solution B (see below) was added in equal volume to the supernatant. The suspension was then transferred to glass centrifuge tubes and centrifuged at 10–14°C for 8 min at 2000 rpm. The cell pellet was washed with solution B, resuspended, and recentrifuged. The initial minced fragments were reincubated with another 40 ml of solution A containing protease and hyaluronidase for 20 min and carried through the above centrifugation and washing procedure. A third incubation of the fragments with 40 ml of solution B was also collected. All pellets were combined, resuspended in solution B, filtered through a 4 × 4 gauze, and respun. The combined cell pellet was then resuspended in choline solution (see below).

Solution A (pH 7.4) consisted of (in m*M*): NaCl, 130.0; $NaHCO_3$, 12.0; Na_2HPO_4, 3.0; NaH_2PO_4, 3.0; K_2HPO_4, 3.0; $MgSO_4$, 2.0; $CaCl_2$, 1.0; glucose, 5.6; and rabbit albumin, 1.0 mg/ml. Solution B (pH 7.4) consisted of (in m*M*): NaCl, 132.4; KCl, 5.4; Na_2HPO_4, 5.0; NaH_2PO_4, 1.0; $MgSO_4$, 1.2; $CaCl_2$, 1.0; glucose, 5.6; and rabbit albumin, 2 mg/ml. Ringer's–Hepes (pH 7.4) solution consisted in m*M* of: NaCl, 136.0; KCl, 5.0; $CaCl_2$, 3.6;

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$MgCl_2$, 2.4, glucose 5.6 and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes), 5.0. Ringer's–Hepes–NH_4Cl (pH 7.4) consisted of Ringer's–Hepes containing NH_4Cl, 20 m*M*. Choline solution was similar to Ringer's–Hepes solution except that the Na^+ and K^+ were replaced with equimolar amounts of choline chloride.

The isolated surface cells were loaded with H^+ using the NH_4^+ prepulse technique previously described (2). Surface cells were incubated in Ringer's–Hepes–NH_4Cl for 30 min at 37°C with 100% O_2. The cells were then centrifuged and resuspended in appropriate solutions. Acridine orange (AO), a metachromatic dye, was used to monitor the relative intracellular pH gradient by the technique of Lee and associates (5, 6). The trapped, charged dye within the cell or the decrease in concentration of external dye is a measure of the difference between the extra- and intracellular pH. Aliquots of cells in the present study were suspended and equilibrated in acridine orange and the fluorescence of the supernatant was measured after rapid pelleting of the cells.

Acid-loaded or control cells were resuspended in choline solution (1–3 ml) and 0.2-ml aliquots were added to 1.4 ml of corresponding solution in microfuge tubes (Beckman) containing AO, 1.25 μM. The cellular suspension was then mixed on a vortex stirrer, allowed to stand 10 min, and finally spun for 60 sec in a Beckman microfuge (Model B). The supernatant was withdrawn and the fluorescence read on an Aminco-Bowman spectrophotometer with excitation at 493 nm and emission at 530 nm. Two-tenths milliliter of appropriate solution added to 1.4 ml of AO solution was carried through the above procedure and used as cell-free blanks to determine initial fluorescence values. Quenching or enhancement of fluorescence was not detected for any of the agents used in this study.

Following resuspension of the acid-loaded cells in choline solution, an initial sample was taken and immediately after that an agent (adjusted to pH 7.4) was added to the cellular suspension. An equal amount of diluent (distilled H_2O) used for the compounds was added to the control cells. Subsequent samples were taken 5, 10, and 20 min later. Arbitrary fluorescence units were calculated by subtracting the fluorescence values of the supernates from the initial fluorescence values of cell-free blanks. A zero value indicates no change in pH_i; an increasing negative value indicates a decrease in pH_i; and a decreasing negative value indicates an increase in pH_i.

The cells used for each individual experiment were obtained from a single rabbit. Thus, *N* refers to the number of rabbits. Control and treated cells for each individual experiment were obtained from the same animal. Statistical analysis of the results included testing the difference between the slopes of the least-squares regression lines for each experimental group and its paired control group of cells. The *P* values given refer to this analysis unless stated to the contrary and a value <0.05 is considered to be significant.

Cimetidine, diphenhydramine, histamine, and pyrilamine were obtained from Sigma Chemical Co. (Saint Louis, MO); 2-(2-aminoethyl)pyridine was obtained from Aldrich Chemical Co. (Milwaukee, WI). Dimaprit and SK&F 93479 were the generous gift of Dr. M. E. Parsons (The Research Institute, Smith Kline and French Laboratories Ltd., Welwyn Garden City, Hertfordshire, England).

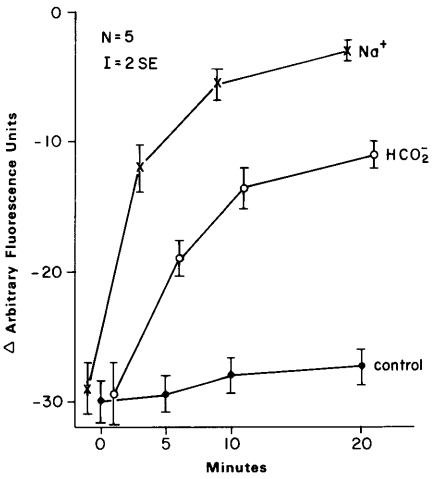

FIG. 1. Spontaneous dissipation of the pH_i and the effects of Na^+ and HCO_3^- on dissipation of pH_i of acid-loaded cells expressed as arbitrary fluorescence units. Aliquots of cells were taken at Time 0 and the subsequent times indicated after no addition or addition of NaCl, 50 m*M*, or choline bicarbonate, 20 m*M*, to cells suspended in choline solution. The slopes of least-squares regression line (5–20 min) for Na^+- and HCO_3^--treated are significantly different from that of the paired control cells ($P < 0.001$).

Results. *Na^+ and HCO_3^-:* The intracellular proton gradient (pH_i) of acid-loaded cells resuspended in choline solution is shown in Fig. 1. A small but significant spontaneous dissipation of the pH_i occurs. Significantly greater dissipation of the pH_i occurs following the addition of either Na^+ or HCO_3^- to paired cells suspended in the same solution, Fig. 1. Similar observations have been reported previously (1–3).

Histamine. Histamine in concentrations of 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} *M* has no significant effect on the spontaneous dissipation of the pH_i ($N = 5$ for each concentration; data not shown). Histamine in the same concentrations also does not significantly affect Na^+-evoked dissipation of the pH_i ($N = 5$ for each concentration; data not shown). In contrast, histamine, 10^{-5} *M*, significantly increases the HCO_3^--evoked dissipation of the pH_i of cells resuspended in choline solution, Fig. 2. The dissipation is progressively greater as the concentration of histamine is increased 10-fold.

Aminoethylpyridine. The H_1 agonist 2-(2-aminoethyl)pyridine (aminoethylpyridine) in concentrations of 10^{-5}, 10^{-4}, and 10^{-3} *M* neither significantly affects the spontaneous ($N = 5$ for each concentration) nor Na^+- ($N = 5$ for each concentration) evoked dissipation of the pH_i (data not shown). Aminoethylpyridine in concentrations of 10^{-5} and 10^{-4} M also does not significantly affect HCO_3^--evoked dissipation of the pH_i. However, aminoethylpyridine in a concentration of 10^{-3} *M* causes a significant increase in HCO_3^--evoked dissipation of the pH_i, Fig. 3.

Pyrilamine. The H_1 receptor antagonist pyrilamine in concentrations of 10^{-6}, 10^{-5}, and 10^{-4} *M* has no significant effect on the spontaneous dissipation of the pH_i ($N = 5$ for each concentration; data not shown). Furthermore, pyrilamine in the same concentra-

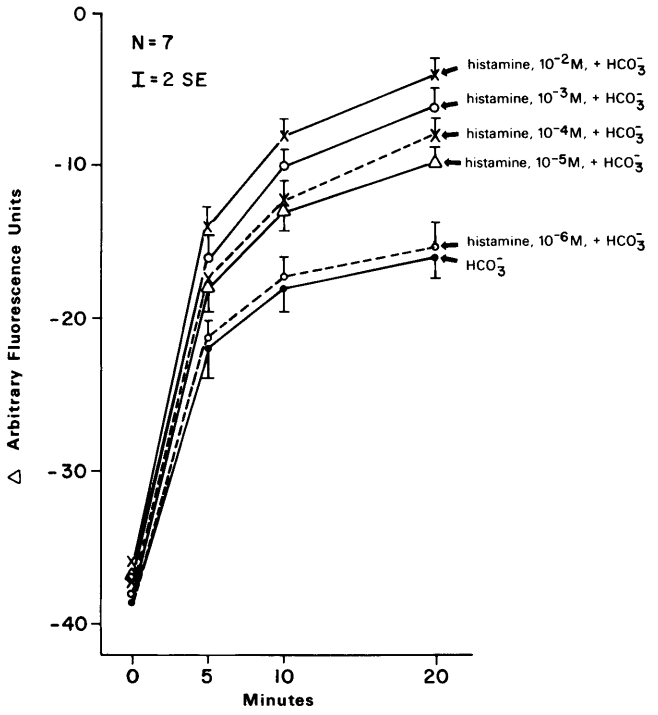

FIG. 2. Effect of increasing concentrations of histamine on HCO_3^--evoked dissipation of pH_i of acid-loaded cells expressed as arbitrary fluorescence units. Aliquots of cells were taken at Time 0 and the subsequent times indicated after the addition of choline bicarbonate, 20 m*M*, and histamine in the concentrations shown to cells suspended in choline solution. Histamine was not added to control cells. The difference between the slopes of least-squares regression lines (5–20 min) for controls (HCO_3^- alone) and concentrations of histamine greater than 10^{-6} M are significant (P at most < 0.01).

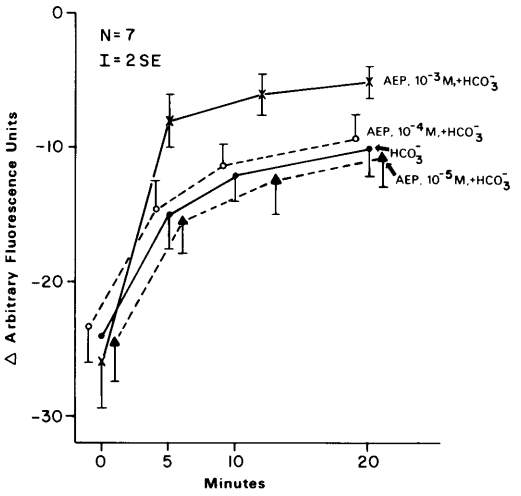

FIG. 3. Effects of aminoethylpyridine (AEP) on HCO_3^--evoked dissipation of the pH_i of acid-loaded cells expressed as arbitrary fluorescence units. Aliquots of cells were taken at Time 0 and the subsequent times indicated after the addition of choline HCO_3^-, 20 m*M*, and AEP in the concentrations shown to cells suspended in choline solution. The control cells (HCO_3^-) were exposed to HCO_3^- only. Only the difference between the slopes of the least-squares regression lines (5–20 min) for 10^{-3} M AEP and control cells is significant ($P < 0.01$).

tions neither significantly affects the Na^+- ($N = 8$ for each concentration) nor HCO_3^-- ($N = 5$ for each concentration) evoked dissipation of the pH_i (data not shown). However, pyrilamine prevents the increase in dissipation of the pH_i that histamine causes in the presence of HCO_3^-, Fig. 4.

Diphenhydramine. Another H_1 receptor antagonist, diphenhydramine, has effects similar to pyrilamine. Diphenhydramine in concentrations of 10^{-6}, 10^{-5}, and 10^{-4} *M* has no significant effect on the spontaneous dissipation of the pH_i ($N = 5$ for each concentration; data not shown). Diphenhydramine in the same concentrations also affects neither Na^+- ($N = 5$ for each concentration) nor HCO_3^-- ($N = 6$ for each concentration) evoked dissipation of the pH_i (data not shown). However, diphenhydramine prevents ($N = 5$, $P < 0.001$) the increase in HCO_3^--evoked dissipation of the pH_i observed in the presence of histamine (data not shown).

Dimaprit. The H_2 agonist dimaprit in concentrations of 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M affects neither spontaneous nor Na^+-evoked dissipation of the pH_i ($N = 5$ or 6 for each concentration in each circumstance; data not shown). Dimaprit in concentrations of 10^{-6}, 10^{-5}, and 10^{-4} M also does not significantly affect HCO_3^--evoked dissipation of the pH_i ($N = 5$ for each concentration; data not shown except for 10^{-4} *M*, Fig. 5). However, dimaprit, like the H_1 agonist aminoethylpyridine, in a concentration of 10^{-3} M, causes a significant increase in HCO_3^--evoked dissipation of the pH_i, Fig. 5.

Cimetidine. The H_2 receptor antagonist cimetidine in concentrations of 10^{-6} and 10^{-5} M does not significantly affect spontaneous dissipation of the pH_i. However, higher concentrations, 10^{-4} ($P < 0.05$) and 10^{-3} *M* ($P < 0.01$), significantly increase spontaneous dissipation ($N = 5$ for each concentration; data for these specific determinations not shown, but see Fig. 6 for 10^{-3} *M*). In spite of this effect, both Na^+- and HCO_3^--evoked dissipation of the pH_i are intact and do not appear to be

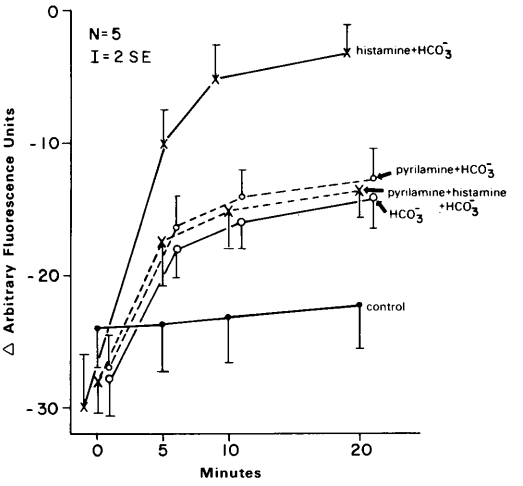

FIG. 4. Effect of pyrilamine, 10^{-3} M, on HCO_3^--evoked dissipation of the pH_i of acid loaded cells in the presence and absence of histamine, 10^{-3} M. pH_i is expressed as arbitrary fluorescence units. Aliquots of cells were taken at Time 0 and the subsequent times indicated after the addition of choline HCO_3^-, 20 m*M*, pyrilamine, and histamine to cells suspended in choline solution. The control cells were not exposed to HCO_3^-. HCO_3^- alone refers to cells exposed to HCO_3^- without pyrilamine or histamine. The difference between the slopes of least-squares regression lines (5–20 min) for histamine and control ($P < 0.001$) as well as histamine and pyrilamine+histamine ($P < 0.001$) are significant.

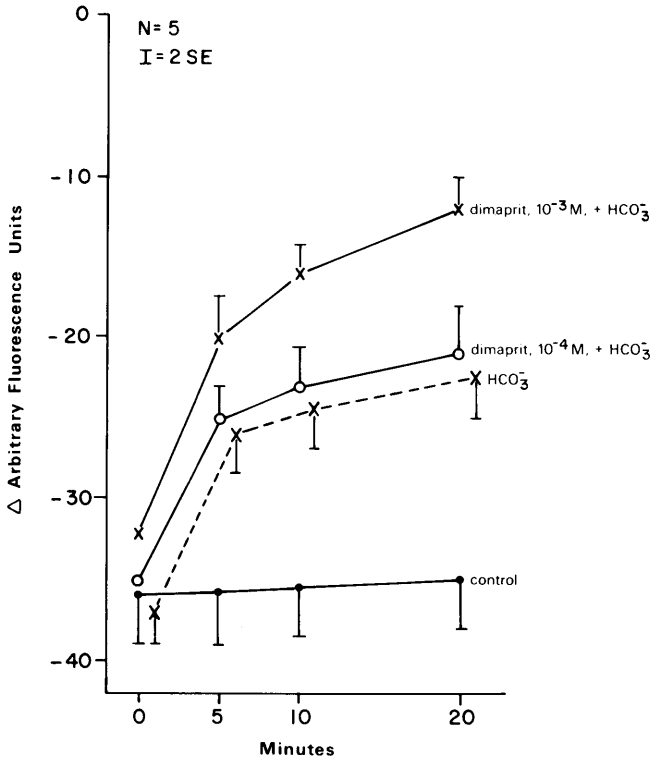

FIG. 5. Effect of dimaprit, 10^{-4} and 10^{-3} M, on HCO_3^--evoked dissipation of the pH_i of acid-loaded cells expressed as arbitrary fluorescence units. Aliquots of cells were taken at Time 0 and the subsequent times indicated after the addition of choline HCO_3^-, 20 m*M*, and dimaprit to cells suspended in choline solution. The control cells were not exposed to HCO_3^-. HCO_3^- alone refers to cells exposed to HCO_3^- without dimaprit or histamine. The slopes of least-squares regression lines (5–20 min) for dimaprit and/or HCO_3^--treated cells are significantly different from control cells (*P* at most <0.01) as are the slopes for dimaprit, 10^{-3} M, (*P* < 0.001) and all other cells groups.

influenced by cimetidine (*N* = 5 for each; data for these specific determinations not shown, but see Fig. 6 for HCO_3^-). Cimetidine, 10^{-4} M, like the H_1 receptor antagonists, also blocks the effect of histamine, 10^{-4} M, on HCO_3^--evoked dissipation of the pH_i, Fig. 6.

Another H_2 receptor antagonist (7), SK&F 93479, in a concentration of 10^{-4} M has effects identical to cimetidine on spontaneous, Na^+-, and HCO_3^--evoked dissipation of the pH_i as well as blocking the effect of histamine on HCO_3^--evoked dissipation (data not shown).

Aminoethylpyridine and dimaprit combined. The combined effects of both histamine agonists on HCO_3^--evoked dissipation of the pH_i are shown in Fig. 7. No effect is evident when the concentrations of both agonists is 10^{-5} M. This concentration as well as 10^{-4} M of each agonist alone as shown in Figs. 3 and 5 is also without effect. However, as long as one of the agonists is present in a concentration of 10^{-4} M and the other is present in a concentration of 10^{-5} M, significant HCO_3^--evoked dissipation of the pH_i occurs.

Discussion. Surface cells isolated from gastric mucosa possess at least two mechanisms for controlling intracellular pH (1, 2). The cells can dissipate a proton gradient by exchanging extracellular Na^+ with intracellular H^+ and exchanging extracellular HCO_3^- with intracellular Cl^-. Histamine does not affect Na^+/H^+ exchange. However, histamine does increase HCO_3^--evoked dissipation of an intracellular proton gradient. It has been shown that such dissipation results from an exchange of extracellular HCO_3^- with intracellular Cl^- (1, 3). Histamine, furthermore, has been reported to reduce Cl^- activity in gastric mucosal surface

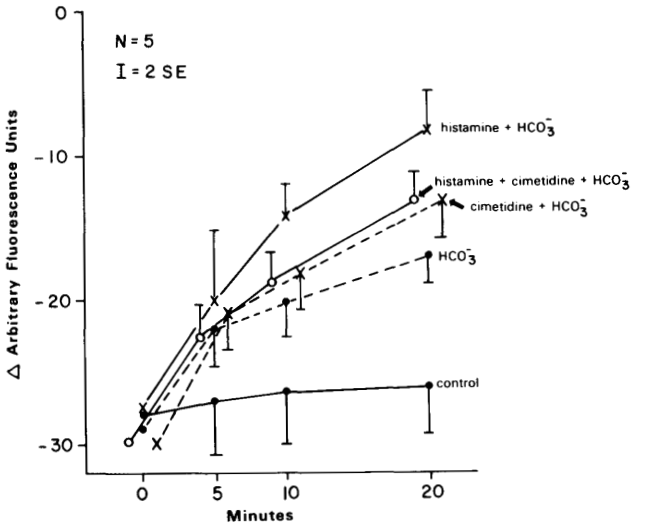

FIG. 6. Effect of cimetidine, 10^{-3} M, on HCO_3^--evoked dissipation of the pH_i of acid-loaded cells in the presence and absence of histamine, 10^{-3} M. pH_i is expressed as arbitrary fluorescence units. Aliquots of cells were taken at Time 0 and the subsequent times indicated after the addition of choline HCO_3^-, 20 m*M*, cimetidine, and histamine to cells suspended in choline solution. The control cells were not exposed to HCO_3^-. The differences between the slopes of least-squares regression lines (5–20 min) for control and all groups of cells are significant ($P < 0.001$). The slopes of least-squares regression lines (5–20 min) are also significantly different between histamine+HCO_3^- and cimetidine+HCO_3^- ($P < 0.05$) and between histamine+HCO_3^- and histamine+cimetidine+HCO_3^- ($P < 0.05$).

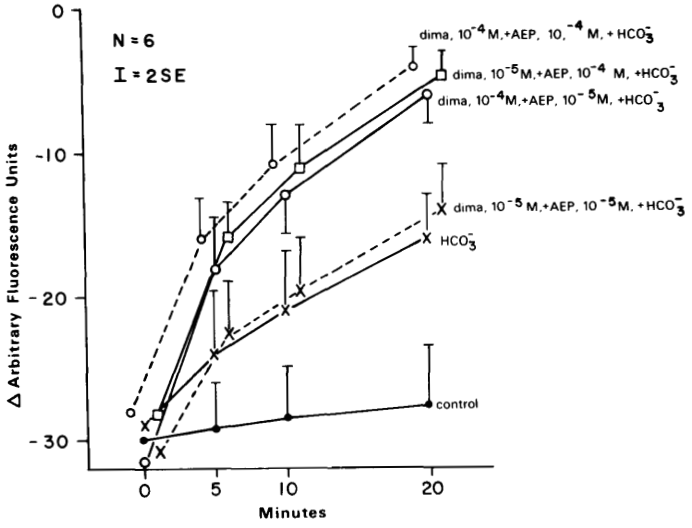

FIG. 7. Effects of combined concentrations of dimaprit (dima) and aminoethylpyridine (AEP) that by themselves are ineffective (See Figs. 3 and 6) in dissipating the pH_i of acid-loaded cells exposed to HCO_3^-. pH_i is expressed as arbitrary fluorescence units. Aliquots of cells were taken at Time 0 and the subsequent times indicated after the addition of choline HCO_3^-, 20 m*M*, dima, and AEP to cells suspended in choline solution. The control cells were not exposed to HCO_3^-. The difference between the slopes of least-squares regression lines (5–20 min) for all groups and control are significant ($P < 0.001$). However, the difference between slopes for HCO_3^- only and dima+AEP at 10^{-5} M + HCO_3^- is not significant. The differences in slopes for all other concentrations for dima+AEP+HCO_3^- and HCO_3^- are significant (P at most <0.01).

epithelial cells (8). The ability of histamine to effect HCO_3^- movement into surface cells can be viewed as part of a coordinated response that helps maintain gastric mucosal integrity during stimulation of H^+ secretion.

It is unlikely that the effect of histamine-causing dissipation of the intracellular proton gradient is a result of H^+ secretion by surface cells. If this were the case, one would expect an effect of histamine on the gradient of acid-loaded surface cells suspended in choline solution with and without Na^+. Clearly, this is not the case. While contamination of the isolated surface cells by parietal cells conceivably could mask the presence or absence of an effect of histamine on surface cells, the present method of isolating surface cells yields a greater than 95% pure population that lacks enzyme activity characteristic of parietal cells (4).

Histamine stimulates H^+ secretion by parietal cells almost exclusively through H_2 receptors. The stimulation of HCO_3^--induced dissipation of a proton gradient in surface cells is more complex. The H_1 agonist aminoethylpyridine (AEP) also enhances HCO_3^--evoked dissipation of the proton gradient. That an H_1 receptor is involved in this effect of histamine is further supported by the observations that the H_1 receptor antagonists pyrilamine and diphenhydramine block the ability of histamine to dissipate the gradient. Additional studies, however, also suggest involvement of an H_2 receptor. The H_2 agonist dimaprit stimulates HCO_3^--evoked dissipation of the gradient and this effect is inhibited by the H_2 receptor antagonists cimetidine and SKF 93479.

There is some degree of spontaneous dissipation of an intracellular proton gradient in the absence of extracellular Na^+ and HCO_3^-. Cimetidine and SKF 93479 appear to increase this spontaneous dissipation but the H_2 agonist dimaprit is without effect in this instance. Even though the H_2 receptor antagonists may alter the accumulation of the dye marker of intracellular pH used in this study or act as an intracellular buffer or possibly effect a nonspecific gradient dissipation, the H_2 antagonists still block the effect of histamine and do not interfere with Na^+- or HCO_3^--evoked dissipation of the pH_i.

While histamine shows a significant effect on HCO_3^--evoked dissipation at a concentration of 10^{-5} *M*, the individual H_1 and H_2 agonists were not effective until use of a concentration of 10^{-3} *M*. However, when AEP and dimaprit were used together, they are able to mimic the effects of histamine at a concentration of 10^{-4} M for both agents or 10^{-4} M for one and 10^{-5} M for the other. This observation supports the concept that the effect of histamine on HCO_3^--evoked dissipation of an intracellular proton gradient is mediated through a coordinated response of both H_1 and H_2 receptors. A coordinated response of H_1 and H_2 receptors mediating an effect of histamine is not unique to surface cells. A similar effect has been reported for the submucosal arterioles of rat stomach, where both H_1 and H_2 receptors mediate vasodilatation (9).

It is well-established that histamine stimulation of acid secretion by parietal cells involves an increase in activity of adenylate cyclase and hence cyclic adenosine monophosphate (cyclic AMP). Stimulation of cyclic AMP by histamine has also been reported to occur in parietal cell poor populations of dispersed gastric mucosal cells (10–12). This effect noted for piglet and guinea pig cells appears to be mediated by an H_1 receptor. However, the effect is controversial because others have observed that adenylate cyclase activity in a dispersed gastric mucosal cell population from guinea pigs is influenced by both H_1 and H_2 receptors (9). In addition to the problems inherent with the use of an impure cellular preparation and possible loss or alteration of receptor sites resulting from the method of cellular preparation, there exist important specie differences in histamine receptors (13). Nevertheless, it is unlikely that the effect of histamine-evoked dissipation of a proton gradient of rabbit surface cells is mediated by cyclic AMP. Exposure of these cells to exogenous dibutyryl cyclic AMP and a phosphodiesterase inhibitor (isobutyl methyl xanthine) impairs the effect of HCO_3^- in dissipating a proton gradient (1).

1. Furukawa T, Olender EJ, Fromm D, Kolis M. Effects of cyclic adenosine monophosphate and prostaglandins on Na^+ and HCO_3^- induced dissipation of a pro-

ton gradient in isolated gastric mucosal surface cells of rabbits. Gastroenterology **89**:500–506, 1985.
2. Olender EJ, Fromm D, Furukawa T, Kolis M. H^+ disposal by rabbit gastric mucosal surface cells. Gastroenterology **86**:698–705, 1984.
3. Umebayashi Y, Olender EJ, Fromm D, Woods DJ. Effects of thiol containing compounds on Na^+/H^+ and Cl^{-/HCO_3^-} exchange in isolated gastric mucosal surface cells of rabbits. (Abstract) Gastroenterology **90**:1673, 1986.
4. Tanaka K, Fromm D, Hill RB, Kolis M. Isolation and viability of gastric mucosal surface cells of the rabbit. J Surg Res **33**:265–279, 1982.
5. Lee HC, Quintanilha AT, Forte JG. Energized gastric microsomal membrane vesicles: An index using metachromatic dyes. Biochem Biophys Res Commun **72**:1179–1186, 1976.
6. Lee HC, Forte JG. A study of H^+ transport in gastric microsomal vesicles using fluorescent probes. Biochim Biophys Acta **508**:339–356, 1978.
7. Blakemore RC, Brown TJ, Durant GJ, Ganellin CR, Parsons ME, Rusmussen AC, Rawlings DA. SK&F 93479, a potent and long acting histamine H_2-receptor antagonist. Brit J Pharmacol **74**:200P, 1981.
8. Curci S, Schettino T, Frömter E. Histamine reduces Cl^- activity in surface epithelial cells of frog gastric mucosa. Pfluegers Arch **406**:204–211, 1986.
9. Guth PH, Moler TL, Smith E. H_1 and H_2 histamine receptors in rat gastric submucosal arterioles. Microvasc Res **19**:320–328, 1980.
10. Batzri S, Gardner JD. Action of histamine on cyclic AMP in guinea pig gastric cells: inhibition by H_1- and H_2-receptor antagonists. Mol Pharmacol **16**:406–416, 1979.
11. Gespach C, Bouhours D, Bouhours J-F, Rosselin G. Histamine interaction on surface recognition sites of H_2-type in parietal and nonparietal cells isolated from the guinea pig stomach. FEBS Letters **149**:85–90, 1982.
12. Rutten MJ, Machen TE. Histamine, cyclic AMP, and activation events in piglet gastric mucosa in vitro. Gastroenterology **80**:928–937, 1981.
13. Hirschowitz BI. An update on histamine receptors and the gastrointestinal tract. Dig Dis Sci **30**:998–1004, 1985.

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