Na^+ Effects on Intracellular pH of Isolated, Perfused Rabbit Gastric Mucosal Surface Cells (43484)

HIRO KOYANAGI, JAN KRANIAK, AKIRA FUROI, PETER JENNINGS, AND DAVID FROMM[1]
Department of Surgery, Wayne State University School of Medicine, Detroit, Michigan 48201

Abstract. **The effects of extracellular Na^+ on intracellular pH were studied by perfusing BCECF loaded gastric mucosal surface cells adherent to glass coverslips held in a spectrophotofluorometer. Removal of Na^+ from a NaCl Ringer perfusate (pH 7.4) resulted in progressive intracellular acidification, which was partially blocked by amiloride. An H^+ conductance did not appear to be present. Acidification induced either by Na^+ removal or by a NH_4 prepulse was reversed by extracellular Na^+, but this effect was not completely prevented by amiloride. Amiloride significantly, but not completely, inhibited Na^{22} uptake by gastric mucosal surface cells. The data suggest that extracellular Na^+ maintains intracellular pH of gastric mucosal surface cells through amiloride-sensitive and -insensitive pathways. In the absence of extracellular Na^+, cellular acidification seemed to be partially due to Na^+/H^+ exchange.** [P.S.E.B.M. 1992, Vol 201]

Previous studies on isolated gastric mucosal surface cells obtained from rabbits suggest a predominant influence of extracellular Na^+ on the regulation of intracellular pH (1, 2). However, the mechanisms of intracellular pH regulation by gastric mucosal surface cells are incompletely understood. Much of the data relating to regulation of intracellular pH by these cells were obtained either by using a pH-sensitive fluorescent dye that binds to cytoplasmic organelles (1) or in the presence of a more appropriate probe, but where dye leakage was difficult to quantitate precisely due to required manipulation of the cells (2, 3). The latter problem is largely obviated in the present study, which employs a method in which surface cells adhere to a glass slide, thus permitting continuous extracellular perfusion of the cells. Using this system, we investigated some of the effects of extracellular Na^+ on intracellular pH.

Methods

Chemicals and Solutions. Chemicals were obtained from the following sources: amiloride HCl, nigericin, valinomycin, and carbonyl cyanide-*m*-chlorophenyl hydrazone (CCCP) were from Sigma; 2,7-bis(2-carboxyethyl)-5(and-6) carboxyfluorescein, and acetoxymethyl ester (BCECF/AM) were from Molecular Probes, Inc. BCECF/AM (1.0 m*M*) in dimethyl sulfoxide and nigericin (10 m*M*) in ethanol were stored up to 30 days at −20°C. Amiloride (100 m*M*) in dimethyl sulfoxide was stored up to 30 days at 5°C.

Phosphate-buffered saline contained: NaCl, 149.6 m*M*; K_2HPO_4, 3.0 m*M*; and NaH_2PO_4, 0.64 m*M*. Enzyme medium (pH 7.4) contained: NaCl, 130 m*M*; $NaHCO_3$, 12.0 m*M*; Na_2HPO_4, 3.0 m*M*; NaH_2PO_4, 3.0 m*M*; K_2HPO_4, 3.0 m*M*; $MgSO_4$, 2.0 m*M*; $CaCl_2$, 1.0 m*M*; dextrose, 5.6 m*M*; hyaluronidase (Type 4; Sigma) 0.4 mg/ml; protease (Type 14; Sigma), 0.05 mg/ml; and rabbit albumin (Sigma), 1.0 mg/ml. Modified phosphate-buffered saline (pH 7.4) contained: NaCl, 132.4 m*M*; KCl, 5.4 m*M*; Na_2HPO_4, 5.0 m*M*; NaH_2PO_4, 1.0 m*M*; $MgSO_4$, 1.2 m*M*; $CaCl_2$, 1.0 m*M*; dextrose, 11.2 m*M*, and rabbit albumin, 2.0 mg/ml. NaCl Ringer solution contained: NaCl, 130 m*M*; KCl, 5 m*M*; $CaCl_2$, 3.6 m*M*; $MgCl_2$, 2.4 m*M*; HEPES, 25 m*M*; and dextose, 5.6 m*M*. NMGCl Ringer was the same as NaCl Ringer, except NaCl was replaced with equimolar concentrations of *N*-methyl-D-glucamide (NMG) and HCl. CCl Ringer was the same as NaCl Ringer, except the Na^+ was replaced with an equimolar concentration of choline chloride. NMG-gluconate Ringer was the same as NaCl Ringer, except the Na^+ was replaced with NMG

[1] To whom requests for reprints should be addressed at Department of Surgery, 6C University Health Center, 4201 St. Antoine, Detroit, MI 48201.

Received December 5, 1991. [P.S.E.B.M. 1992, Vol 201]
Accepted April 20, 1992.

0037-9727/92/2011-0088$3.00/0

gluconate and D-gluconic acid lactone and the other Cl^- components were replaced by equimolar concentrations of the respective gluconate replaced by equimolar concentrations of the respective gluconate compounds. NH_4 Ringer is the same as NaCl Ringer, except 30 m*M* of NaCl was replaced with equimolar NH_4Cl. KCl Ringer was the same as NaCl Ringer, except Na^+ was replaced with an equimolar concentration of K^+.

The pH of all solutions was adjusted to 7.4 with NMG base. All solutions were bubbled with 100% O_2 for 20 min before use and during cellular perfusion in order to remove CO_2. The osmolality of all solutions was between 280–290 mOsm.

Isolation of Gastric Mucosal Surface Cells. Gastric mucosal surface cells from male New Zealand White rabbits were prepared by a modification of the method of Tanaka *et al.* (4). Rabbits were anesthetized with ketamine (40 mg/kg im), xylazine (5 mg/kg im), and pentobarbital (50 mg iv). The stomach was removed after it was perfused with phosphate-buffered saline (500 ml), at 37°C through the aorta in order to wash out blood. The mucosa of the fundus and body was scraped from its muscularis, minced with scissors, and incubated in enzyme medium for 45 min at 37°C with constant stirring. The digest was filtered through gauze and centrifuged at 4°C at 1300*g* for 8 min.

The cells, suspended in modified phosphate-buffered saline, were placed in self-generated, isopycnic density gradients of Percoll (5) in order to minimize contamination with other glandular, red blood, dead, and fragmented cells. The cell suspension was layered on a 45% (v/v) isotonic Percoll cushion in modified phosphate-buffered saline (300 mOsm; initial density, 1.059) and centrifuged at 4°C for 20 min at 30,000*g* with a 34° angle head rotor. The surface cell fraction (density, 1.056 ± 0.002, $n = 16$) was collected and the cells were separated from Percoll medium by washing and centrifugation with modified phosphate-buffered saline (5 vol to 1 vol of cell suspension) at 1,300*g* for 8 min. The purified surface cells displayed greater than 95% viability as judged by erythrosine B dye exclusion at the end of each individual experiment.

Histologic studies of the isolated cells were performed as reported previously and identical results were observed (4). The surface cell preparation contained less than 5% red blood and glandular cells. Surface cells are readily identified because they contain dense, coarse, PAS-positive material (Fig. 1, a, d, e, and f). Zymogen cells also contain PAS-positive material, but it has a stippled appearance (Fig. 1 b and c).

BCECF Loading. The cells were placed in modified phosphate-buffered saline containing 4.0 μM BCECF/AM (6–8) for 30 min at 37°C and then washed free of extracellular dye with modified phosphate-buffered saline solution by centrifugation at 4°C for 8 min at 1300*g*. The cells, resuspended in the buffer, were divided into two groups, one of which was centrifuged again and washed a third time in NaCl Ringer to remove albumin. The other cell group was kept at room temperature until ready for use.

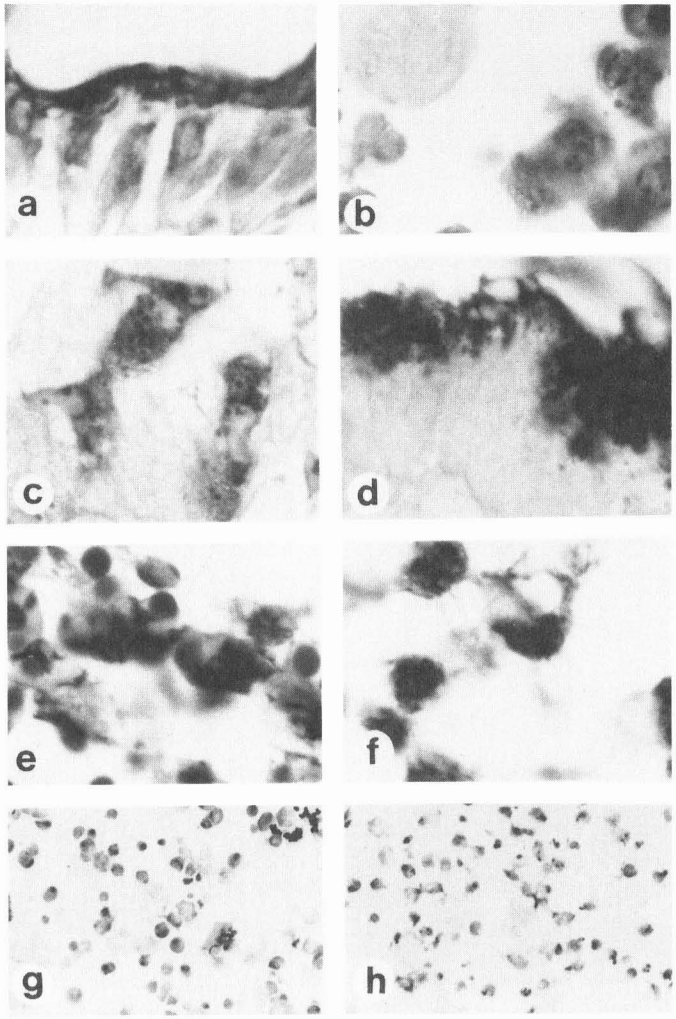

Figure 1. Microphotographs of isolated cells (×750). (a) Periodic acid-Schiff (PAS)-hematoxylin (H)-stained (by method given in Ref. 4) intact mucosa showing dense accumulation of PAS in surface cells. (b) PAS-H-stained isolated gastric glandular cells (prepared as described in Ref. 4) showing seemingly stippled accumulation of PAS in zymogen cells. (c) PAS-H-stained section of intact mucosa showing stippled-appearing accumulation of PAS in zymogen cells. (d) Alcian blue-PAS-stained (by method given in Ref. 4) intact mucosa showing dense accumulation of PAS in surface cells. (e) PAS-H-stained isolated surface cells showing dense accumulation of PAS in cytoplasm. (f) Alcian blue-PAS-stained isolated surface cells showing dense PAS accumulation within the cells. (g) Hematoxylin and eosin stain of polylysine-coated coverslip containing cells at the beginning of a proposed experiment. (h) Hematoxylin and eosin stain of polylysine-coated coverslip at the end of an actual 40-min experiment.

Perfusion of Cells. The cell pellet, resuspended in 200 μl of NaCl-Ringer, was floated onto a polylysine-coated glass coverslip (9) and allowed to settle for 5 min. Coating with polylysine was necessary to enable cells to stick to glass (Fig. 1, g and h). The coverslip holding the cells was placed diagonally in a cuvette located in the thermostated compartment (22°C) of an

SLM spectrophotofluorometer (SPF-500C) so that the coverslip was aligned 45° to the excitation beam (10). The various perfusion solutions, kept in syringes, were delivered to the cuvette by gravity via Teflon or polyethylene tubing. New (previously unused) tubing was used for each experiment. Perfusion velocity, which was regulated by changing the syringe height, was adjusted so that the cuvette volume (2 ml) was exchanged every 15 sec (P. A. Negulescu and T. E. Machen, personal communication).

Measurement of BCECF Fluorescence and Conversion to Intracellular pH. The fluorescence excitation intensity ratio, subsequently converted to intracellular pH (pH_i), was calculated as the 506-nm (pH sensitive) measurement divided by the 436-nm (isobestic) measurement (band width, 5.0 nm), with the fixed emission wavelength at 525 nm (band width, 2.5 nm). The pH-sensitive excitation wavelength was determined by running excitation spectrums at pH_i 6.8, 7.0, 7.3, and 7.5, and pH_i was equilibrated with external pH (pH_o) by using nigericin.

Fluorescence measurements to determine pH_i were taken at 1-min or 2-min intervals. All results were corrected by subtracting background fluorescence emitted from a blank coverslip perfused with the appropriate solution in a reference cuvette. Sham-loaded cells (treated identically as BCECF-loaded cells, but without exposure to BCECF/AM) were found to make an insignificant contribution to background. The fluorescence signal from dye-loaded cells exceeded background by 10- to 50-fold at both 506 and 436 nm.

In order to define the relationship between the fluorescence excitation intensity ratio and pH_i, dye-loaded cells were perfused with KCl Ringer containing 10 μM nigericin (a K+/H+ ionophore) at a minimum of three different pH_o values. Calibration was performed at the end of each experiment. The fluorescence excitation intensity ratio varied linearly with pH_i over a pH_o range of 6.3–7.8, where regression coefficients for calibration data were never <0.985.

Several preliminary studies indicated that nigericin adheres to and leaches from some types of tubing and possibly glass. For this reason, tubing (polyethylene or Teflon) never exposed to nigericin was used for each experiment. Furthermore, all tubing, cuvettes, and other glassware associated with this study were rinsed with ethanol followed by 0.01% Triton X and 2% nitric acid before use. A separate set of tubing was reserved for use with nigericin only.

^{22}Na Uptake. Surface cells, acidified by incubation in CCl Ringer (pH_i < 6.75) for 10 min at 22°C, were exposed to 2 μCi/ml of ^{22}Na and 20 mM Na^+. Aliquots (200 μl) of the suspension were taken after various times of incubation (0, 2, 5, and 10 min), layered on a 0.3 M sucrose gradient, and microfuged for 30 sec in order to separate the cells from extracellular medium and finish the uptake by the cells. The lower part of the microfuge tube including the cell pellet was cut by blade and counted to measure intracellular ^{22}Na using a Packard Auto-Gamma Scintillation Spectrometer. The background ^{22}Na uptake corresponding to Time 0 was measured in the presence of amiloride, either 0.4 or 1.0 mM, at 4°C and subtracted from each data point.

Statistics. A single rabbit provided cells for two or, at most, three individual experiments. Statistical analyses were performed by testing the difference between slopes of least squares regression lines and by using Student's t test for paired and unpaired samples.

Results

The pH_i of surface cells after perfusion for 4 min with NaCl Ringer was 7.18 ± 0.02 and did not change significantly over the next 6 min (Fig. 2A). This was in contrast with cells initially perfused with NaCl Ringer and then with Na^+-free solution (NMGCl Ringer) (Fig. 2B). The pH_i in this situation progressively decreased, even though pH_o was still 7.4. However, the pH_i returned to nearly its baseline level during reperfusion with NaCl Ringer. Subsequent perfusion of the cells with Na^+-free Ringer containing the Na^+ channel blocker amiloride resulted in a lesser ($P < 0.001$), but still significant, decline in pH_i compared with the absence of amiloride (Fig. 2B). Initial perfusion of cells with NaCl Ringer and then replacing Na^+ mole for mole with K^+ did not appear to affect the decline in pH_i (Fig. 2C).

Amiloride inhibited the effect of Na^+ on the pH_i of cells allowed to acidify (Fig. 3A). Upon changing from a Na^+-containing to a Na^+-free perfusate, the expected decline in pH_i occurred. However, the pH_i remained depressed upon subsequent exposure of the cells to NaCl Ringer containing amiloride. Once amiloride was removed from the Na^+-containing perfusate, the pH_i increased, returning to its baseline value.

More rapid acidification occurred using the NH_4 prepulse method (Fig. 3B). Addition of NH_4Cl to the NaCl Ringer perfusate caused expected alkalinization, and removal of NH_4 from the perfusate caused expected, rapid intracellular acidification. The pH_i then increased toward its baseline level, but this recovery was not complete over a 13-min interval.

Amiloride does not completely prevent the effect of Na^+ increasing the pH_i of cells acidified by removal of NH_4 (Fig. 3B). The pH_i remains depressed when acidified cells are subsequently perfused with Na^+-free solution containing amiloride. However, the pH_i increased to some degree when the perfusate was changed to NaCl Ringer containing amiloride, but the increase certainly was not as much as that observed for acidified cells perfused with amiloride-free NaCl Ringer.

That a proton conductance does not significantly contribute to the amiloride-insensitive effect of Na^+ on

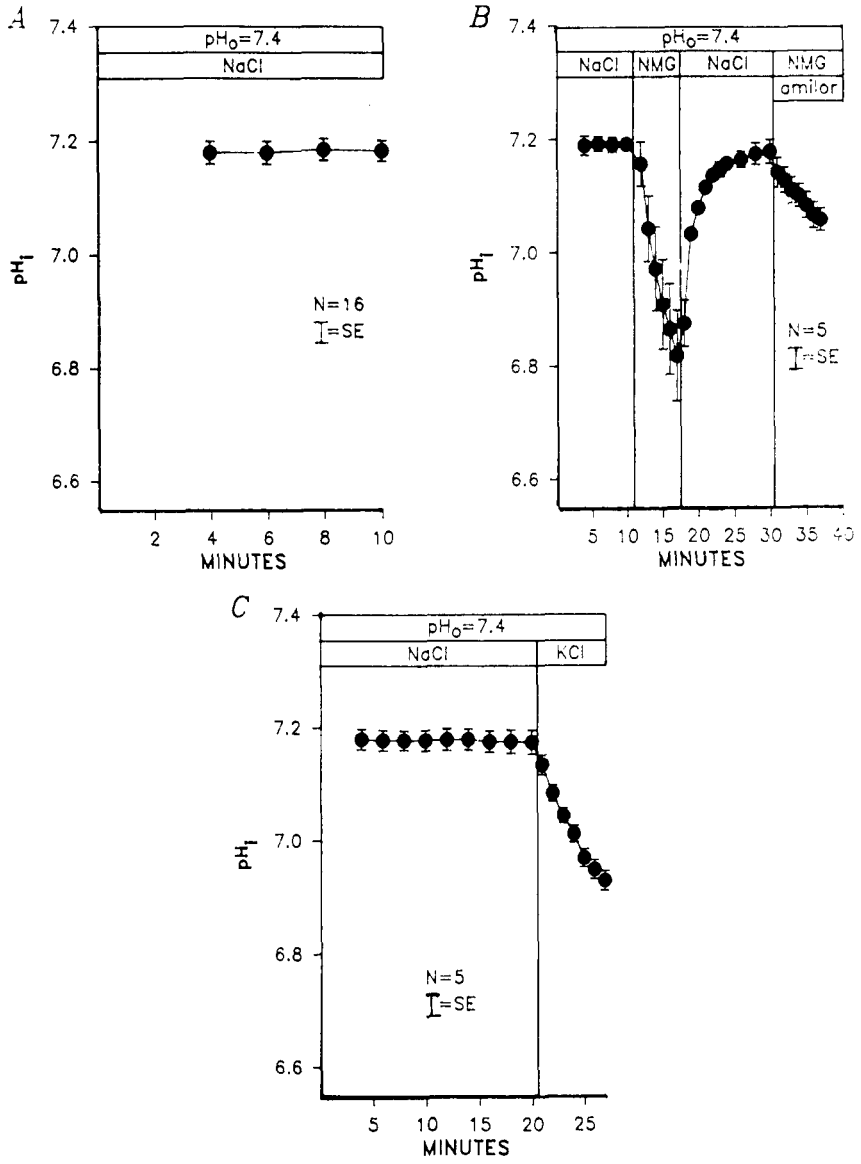

Figure 2. Effects of Na^+, amiloride, and K^+ on pH_i at external pH 7.4. (A) Steady state pH_i of surface cells is reached by 4 min during perfusion with NaCl Ringer (NaCl). Cells used for this experiment were independent of other experiments. (B) Cells initially perfused with NaCl Ringer (NaCl) were subsequently perfused with a Na^+-free solution, NMGCl Ringer (NMG), which resulted in a progressive decline in pH_i. Replacement of Na^+ in the perfusate (NaCl) caused the pH_i to return toward its baseline. Subsequent removal of Na^+ (NMG) with simultaneous addition of 1.0 m*M* amiloride (amilor) resulted in a decrease in pH_i, but the slope of decline of the least squares regression line (31–37 min: $y = 7.588 - 0.014 * x$, $r = 0.997$) was less ($P < 0.001$) than that observed in the absence of amiloride (11–17 min: $y = 7.886 - 064 * x$, $r = 0.988$). (C) Cells initially perfused with NaCl Ringer were subsequently perfused with KCl Ringer (KCl). The slope of decline of the least squares regression line for pH_i during perfusion with KCl (21–27 min: $y = 7.841 - 0.034 * x$, $r = 0.990$) does not significantly differ from the slope of decline observed during perfusion with NMG alone (11–17 min), as shown in Figure 2B.

pH_i is suggested by Figure 3C. Acidified cells, continuously exposed to amiloride, were perfused with NMGCl Ringer and subsequently with KCl Ringer. Presumably, the membrane potential was reduced to zero by the addition of valinomycin to the K^+-containing perfusate, but the pH_i did not significantly change. However, the subsequent addition of the protonophore CCCP caused rapid dissipation of the pH gradient to the point where pH_i equaled pH_o.

Isotopic studies were done to determine the effect of amiloride on Na^+ uptake by surface cells. ^{22}Na uptake occurred after the addition of Na^+ to cells acidified by suspension in a Na^+-free Ringer solution. The uptake of ^{22}Na was significantly inhibited ($P < 0.001$), but not totally prevented, by amiloride (Fig. 4).

Discussion

Isolated gastric mucosal surface cells perfused with pH 7.4 NaCl Ringer solution have a "steady state" pH_i near 7.18 (Fig. 2). In comparison, various studies of surface cells taken from the gastric mucosa report values for pH_i that range from 7.02 to 7.12 (2, 11–14). Such

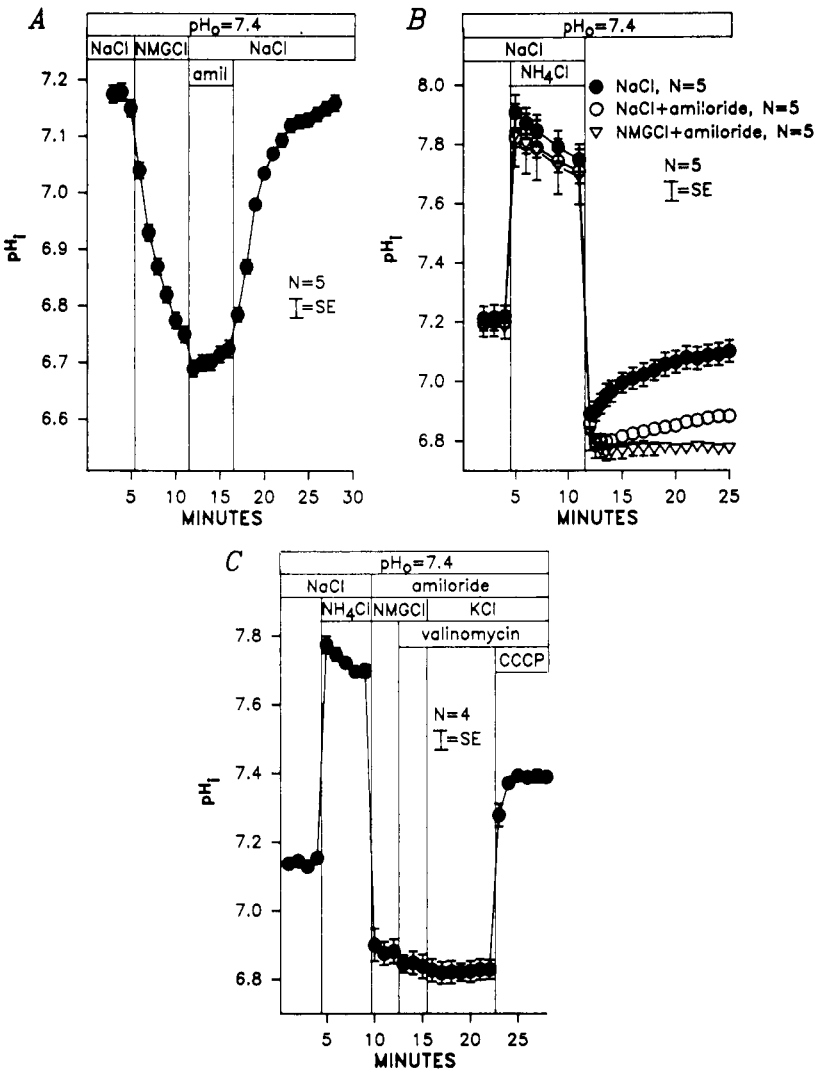

Figure 3. (A) Effect of amiloride on acidified cells at external pH 7.4. Cells were initially perfused with NaCl Ringer (NaCl) and then allowed to acidify during perfusion with an Na^+-free solution, NMGCl Ringer (NMGCl). The cells were subsequently perfused with NaCl Ringer containing 1.0 m*M* amiloride (amil) and the pH_i remained depressed. However, upon removal of amiloride from the NaCl Ringer perfusate, the pH_i returned to its baseline. (B) Effect of amiloride on acidified cells at external pH 7.4. Cells were initially perfused with NaCl Ringer and then perfused with NH_4 Ringer (NaCl Ringer with 30 m*M* NaCl replaced with equimolar NH_4Cl). During exposure to NH_4, the pH_i, as expected, increased due to the influx of NH_3 and then gradually declines as a result of the passive influx of NH_4. The cells rapidly acidified upon removal of NH_4 from the perfusate, as the efflux of NH_3 left H^+ trapped in the cells (17). The pH_i remained depressed upon removal of NH_4 and perfusion with Na^+-free solution (NMGCl Ringer) (NMGCl) containing 1.0 m*M* amiloride. In contrast, the pH_i returned toward its preacidification level upon removal of NH_4 from the NaCl Ringer perfusate. However, this effect of Na^+ was not completely prevented by amiloride. (C) Effects of valinomycin and carbonyl cyanide-m-chlorophenyl hydrazone (CCCP) on cells acidified by NH_4 prepulse at pH_o 7.4. Cells initially perfused with NaCl Ringer were acidified as described in Figure 3B. Amiloride (1.0 m*M*) was added to subsequent perfusates. Neither NMGCl Ringer (NMGCl) without and then with valinomycin (50 μM) nor subsequent perfusion with KCl Ringer (KCl) containing valinomycin caused pH_i to change significantly. However, the addition of CCCP (100 μM) to the KCl Ringer-valinomycin perfusate caused complete dissipation of the pH gradient.

differences may be attributed to specific methods for measurement of pH_i as well as variations in species and the technique of cell preparation. However, rabbit gastric mucosal cells appear to have a reasonably similar pH_i that is reported to be 7.16 for parietal cells, 7.23 for chief cells, and 7.10 for antral cells during exposure to NaCl Ringer (11).

Maintenance of the pH_i was dependent upon extracellular Na^+. Removal of this cation (at pH_o 7.4) resulted in progressive intracellular acidification that was reversed upon readdition of Na^+ (Figs. 2B and 3A). A portion of this acidification was blocked by amiloride (Fig. 2B), a known inhibitor of Na^+/H^+ exchange. Qualitatively similar results have also been reported to occur in rabbit gastric mucosal surface cells isolated by a different method and suspended in various solutions (2). Thus, it appears that there is a bidirectional exchange of Na^+ with H^+, depending upon the Na^+ gradient. The remaining cause(s) of acidification was not clear, but it does not appear to be due to a H^+ conductance (Fig. 3C).

We have observed previously an effect of K^+ on

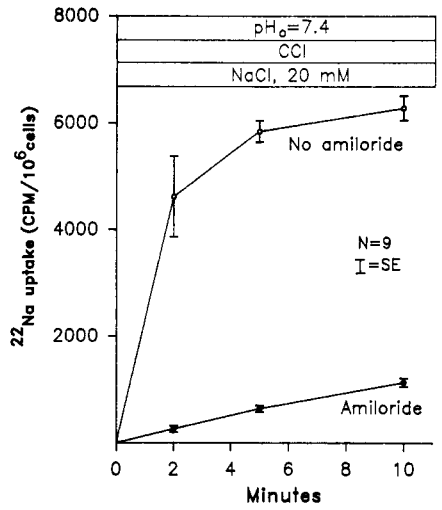

Figure 4. Cumulative ^{22}Na uptake in the presence of 20 m*M* Na^+ by cells acidified by suspension in a Na^+-free solution (CCl Ringer, pH_o 7.4). The pH_i in this situation reaches a nadir of 6.75 ± 0.021 (n = 10, data not shown). ^{22}Na uptake was measured as described in Methods. One of the cell pairs was exposed to amiloride, at either 0.4 m*M* (n = 5) or 1.0 m*M* (n = 4), and the other was not. Since the effects of the two concentrations were not significantly different, the results were combined. Amiloride caused significant ($P < 0.001$), but not complete, inhibition of ^{22}Na uptake.

the pH_i of similarly prepared, isolated gastric mucosal surface cells (15). However, it became apparent that the cellular perfusion apparatus is easily contaminated with nigericin used to calibrate the pH_i at the end of each experiment. Taking strict precautions to avoid such contamination in the present study, it was found that acidification is unaffected by the presence of K^+ in the cellular perfusate (Fig. 2C). Thus, K^+ cannot substitute for Na^+ in maintaining pH_i.

More rapid acidification of surface cells occurred by first exposing cells to and then removing NH_4 (Fig. 3). Acidification by this means was also reversed by the presence of Na^+ in the perfusate (Fig. 3B). One might expect amiloride to block this effect of extracellular Na^+. However, amiloride caused only partial inhibition of the effect of Na^+ on the pH_i of acidified cells (Fig. 3 A and B). While amiloride caused significant inhibition of isotopic Na^+ uptake by surface cells, this inhibition was not absolute (Fig. 4), implying the presence of an amiloride-insensitive Na^+ pathway. An amiloride-sensitive and -insensitive pathway involving Na^+/H^+ exchange has also been suggested to occur in thymic lymphocytes (16) and other cells.

The present data for isolated surface cells come from a perfusate nominally free of HCO_3^-. It is possible, as suggested for other systems, that differences in extracellular buffering by HCO_3^- and HEPES (used in the present experiments) could elicit changes in anion transport (18). Although the present experiments do not address this possibility, the concentration of HEPES was identical in all of the experiments. Thus, it appears that isolated gastric mucosal surface cells in a nominally free HCO_3^- system primarily require Na^+ in order to regulate their pH_i. The effect of extracellular Na^+ is predominantly amiloride sensitive but an insensitive component also exists.

The authors thank Karen Emanuel and Ben True for histological and photomicrographic preparation.

1. Olender EJ, Fromm D, Furukawa T, Kolis M. H^+ disposal by rabbit gastric mucosal surface cells. Gastroenterology **86**:698–705, 1984.
2. Seidler U, Carter K, Ito S, Silen W. Effect of CO_2 on pH_i in rabbit, parietal, chief and surface cells. Am J Physiol **256**:G466–G475, 1989.
3. Furoi A, Jennings P, Fromm D. Effect of H^+ on Na^+/H^+ exchange by rabbit gastric mucosal surface cells. Gastroenterology **96**:A164, 1989.
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. Pertof TH, Laurent TC. Sedimentation of cells in colloidal silica (Percoll). In: Pretlow TG, Pretlow TP, Eds. Cell Separation: Methods and Selected Application. New York: Academic Press, pp115–52, 1982.
6. Kurtz I, Golchini K. Na+-independent $Cl^-HCO_3^-$ exchange in Madin-Darby canine kidney cells. J Biol Chem **262**:4516–4520, 1987.
7. Paradiso AM, Tsien RY, Machen TE. Na^+-H^+ exchange in gastric glands as measured with cytoplasmic-trapped, fluorescent pH indicator. Proc Natl Acad Sci USA **81**:7436–7440, 1984.
8. Thomas JA, Buchsbaum RN, Zimniak A, Racker E. Intracellular pH measurements in Ehrlich ascites tumor cells utilizing spectroscopic probes generated in situ. Biochemistry **18**:2210–2218, 1979.
9. Wenzel E, Machen TE. Intracellular pH dependence of buffer capacity and anion exchange in the parietal cell. Am J Physiol **257**:G741–G747, 1989.
10. Ohkuma S, Poole B. Fluorescence probe measurement of the intralysosomal pH in living cells and the perturbation of pH by various agents. Proc Natl Acad Sci USA **75**:3327–3331, 1978.
11. Townsley MC, Paradiso AM, Machen TE. Na/H exchange in antral cells isolated from *Necturus*, rabbit and guinea pig. Gastroenterology **95**:302–310, 1988.
12. Carter KJ, Saario I, Seidler U, Silen W. Effect of pCO2 on intracellular pH in vitro frog gastric mucosa. Am J Physiol **256**:G206–G213, 1989.
13. Ashley SW, Soybell DI, Cheung LY. Measurements of intracellular pH in Necturus antral mucosa by microelectrode technique. Am J Physiol **250**:G625–G632, 1986.
14. Kivilaakso E, Kiviluoto T. Intracellular pH in isolated Necturus antral mucosa in simulated ulcerogenic conditions. Gastroenterology **95**:1198–1205, 1988.
15. Koyanagi H, Kraniak J, Fromm D. K^+ increase intracellular pH (pH_i) of isolated rabbit gastric mucosal surface cells. Gastroenterology **100**:A693, 1991.
16. Grinstein S, Goetz JD, Rothstein A. ^{22}Na flux in thymic lymphocytes. I. Na/Na and Na/H exchange through an amiloride-insensitive pathway. J Gen Physiol **84**:565–584, 1984.
17. Roos A, Boron WF. Intracellular pH. Physiol Rev **61**:296–434, 1981.
18. Yanaka A, Carter KJ, Goddard PJ, Silen W. Effect of luminal acid on intracellular pH in oxynticopeptic cells in intact frog gastric mucosa. Gastroenterology **100**:606–618, 1991.