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Salicylate Effects on Proton Gradient Dissipation by Isolated Gastric Mucosal Surface Cells[1] (42402)

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Abstract. The effects of salicylate were examined on Na^+/H^+ exchange by isolated gastric mucosal surface cells loaded with H^+ and resuspended in a buffered medium. Choline salicylate (pH 7.4) increases the dissipation of an intracellular proton gradient which was measured using acridine orange. The exchange of extracellular Na^+ with intracellular H^+ by surface cells not only remains intact but also is enhanced upon exposure to salicylate. This was confirmed by cellular uptake of ^{22}Na and titration of cellular H^+ efflux. Salicylate increases Na^+/H^+ exchange via a pathway predominantly sensitive to amiloride. However, the data also suggest that salicylate dissipates an intracellular proton gradient by an additional mechanism. The latter is independent of extracellular Na^+ and not due to a generalized increase in cellular permeability.

Salicylate is known to alter ion fluxes of the gastric mucosa and these changes are usually associated with the damaging effects of the drug. A generalized increase in ion permeability of intact gastric mucosa occurs when salicylate is present in the lumen containing acid (1–7). The resulting enhanced diffusion of H^+ into the mucosa is believed to contribute to cellular damage. One of the earliest detectable anatomic lesions associated with salicylate is the destruction of gastric mucosal surface cells (8). It has recently been established that one mechanism whereby surface cells can regulate their internal pH involves an exchange between extracellular Na^+ with intracellular H^+ (9). The effects of salicylate on this mechanism are unknown.

It is generally assumed that the damaging effects of salicylate in the presence of luminal acid are associated with increasing acidification of the surface cells. This can occur as a result of cellular uptake of the undissociated form of salicylic acid or as a result of increased cellular permeability to the luminal proton gradient. These circumstances would make it exceedingly difficult in an experimental situation to dissociate the effects of an extracellular source of acid from the effects of salicylate alone. In this study, therefore, the effects of choline salicylate were determined on Na^+/H^+ exchange by isolated surface cells that were preloaded with acid and resuspended in a nonacidic medium.

Methods. New Zealand white rabbits (2–4 kg) were anesthetized with xylazine, 5 mg/kg, and ketamine, 40 mg/kg im, and pentobarbital, 1 ml iv, prior to removing the stomach. At least a 90% pure fraction of surface cells was obtained as previously described (9, 10). Briefly, the minced mucosal fragments were incubated in solution A (see below) containing protease, 0.01% (wt/vol), 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 according to the individual experimental protocols.

Solutions A (pH 7.4) consisted of (in m*M*) NaCl, 130.0; $NaHCO_3$, 12.0; Na_2PHO_4, 3.0; NaH_2PO_4, 3.0; K_2HPO_4, 3.0; $MgSO_4$, 2.0;

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$CaCl_2$, 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 of (in m*M*) NaCl, 136.0; KCl, 5.0; $CaCl_2$, 7.2; $MgCl_2$, 2.4; glucose, 5.6; and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), 5.0, Ringer's–Hepes–NH_4Cl (pH 7.4) consisted of Ringer's–Hepes containing NH_4Cl, 20 m*M*. Choline chloride solution was the same as Ringer's–Hepes solution except that 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 (9). 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 (9, 11, 12). 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 the 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 cells were resuspended in appropriate solutions (1–3 ml) and 0.2-ml aliquots were added to 1.4 ml of corresponding solutions 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 maximal initial fluorescence values. Quenching or enhancement of fluorescence was not detected for salicylate. However, slight autofluorescence was observed with 4-acetamido-4′-isothiocyanostilbene-2,2′ disulfonic acid (SITS; Sigma Chemical Co.) and 4-4′diisothiocyanostilbene-2,2 disulfonic acid (DIDS; Pierce Chemical Co.) and appropriate corrections were made. Concentrated stock solutions of these stilbenes were prepared in Tris buffer and diluted in choline chloride to achieve the appropriate experimental concentrations.

The data for intracellular proton gradient are reported as percentage dissipation of the gradient. The difference in fluorescence between that observed for the samples taken at various time points and the initial cellular aliquot was used to determine the intracellular proton gradient. The ratio of the intracellular gradient to the initial gradient was used to determine the percentage change in arbitrary fluorescence units, or percentage dissipation of the intracellular proton gradient. The initial gradients are not presented in the figures showing this dissipation, since the initial gradient was designated as zero dissipation in order to perform statistical analyses. However, the initial gradients ranged from 30 to 56 arbitrary fluorescence units less than the fluorescence value of the same suspension medium without cells.

The changes in intracellular proton gradient are relative, as the actual magnitude of the pH gradient is not known. Furthermore, our use of the term intracellular pH does not specify the subcellular compartment or compartments monitored by acridine orange. The changes in acridine orange fluorescence may reflect pH changes in several intracellular compartments, such as cytoplasm, mitochondria, lysosomes, etc. However, we have previously indicated tht the rapid nature of H^+ efflux in response to Na^+ suggests that a major component of this efflux is located near the cell surface membrane and quickly responds to activation of the Na^+/H^+ exchange system (9).

^{22}Na uptake experiments were performed using acid-loaded cells preincubated at 22°C in Na^+- and K^+-free Ringer's–Hepes (choline chloride) solution containing ouabain, 2 m*M*, and when appropriate, choline salicylate, 5 m*M*. After 5 min, ^{22}Na and 1 m*M* Na^+ were added to the cells. Aliquots were taken just prior to and 5 and 10 min after the addition of Na^+. The samples were filtered on a vacuum manifold apparatus and the filters were counted in a gamma scintillation counter. Al-

iquots containing ^{22}Na but no cells were filtered to determine filter retention of ^{22}Na and these values were subtracted from the cellular uptake values. Total cellular protein was measured by the method of Lowry and associates (13).

For titration of H^+ efflux, acid-loaded cells were resuspended in choline chloride and stirred in a titrator cup. The pH was monitored using a Radiometer PHM62 pH meter and the pH was maintained constant by addition of KOH, 0.01 *N*, with a Radiometer ABU80 autoburette.

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 rabbit. Statistical analyses 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 as well as Student's *t* test for paired variates.

Results. Neubauer chamber counts of acid-loaded surface cells resuspended in choline chloride before ($3.1 \pm 0.3 \times 10^7$) and after 40 min exposure to 10 m*M* choline salicylate ($2.9 \pm 0.4 \times 10^7$, $N = 5$) did not significantly differ. Furthermore, the acid-loaded cells continue to exclude the vital dye trypan blue ($N = 5$) after a 40-min exposure to either 5 or 10 m*M* choline salicylate (hereafter referred to as salicylate) in the choline chloride suspension medium (pH of 7.4).

An intracellular proton gradient (pH_i) is evident when the cells preincubated in NH_4Cl are resuspended in choline chloride, Fig. 1. There is a slow spontaneous dissipation of the pH_i. However, dissipation of the pH_i increases significantly after the addition of salicylate, Fig. 1. This effect is concentration dependent. After the addition of a low concentration of salicylate, 2.5 m*M*, dissipation of the pH_i is greater than paired controls, but the difference does not reach statistical significance until 20 min ($P < 0.05$, and at 30 min, $P < 0.001$) after addition of the drug. Doubling the concentration of salicylate, 5 m*M*, results in significant dissipation of the pH_i beginning at 5 min ($P < 0.05$) and this difference is sustained 30 min ($P < 0.005$) after exposure to the drug. A further doubling of the concentration of salicylate, 10 m*M*, results in a progressively increasing dissipation of pH_i, which is significant 3 min ($P < 0.05$) and also sustained 30 min ($P < 0.001$) later.

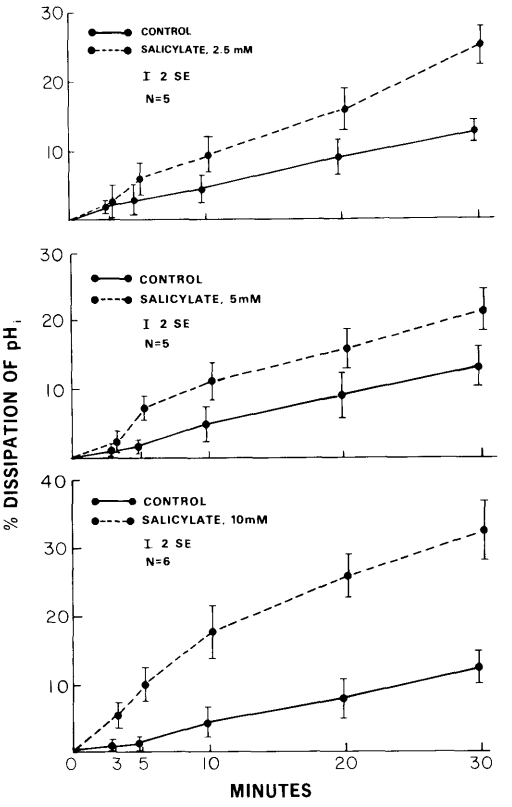

FIG. 1. Effects of increasing concentrations of choline salicylate on dissipation of the intracellular proton gradient (pH_i) of acid-loaded surface cells resuspended in choline chloride. Salicylate was added at 0 time. Only choline chloride solution was added at 0 time to the paired group of control cells. Aliquots of the cell suspension were withdrawn at the indicated times and the percentage dissipation of the pH_i was calculated as described under Methods.

It is possible that the effects of salicylate shown in Fig. 1 are artifacts due to the salicylate interfering with the fluorescence of AO. Even though salicylate does not interfere with AO activity in cell-free solution, additional experiments were done. These involved pH stat titration of choline chloride (without Hepes, pH 7.0) suspension medium containing acid-loaded cells exposed to salicylate (Fig. 2). Although there is a mean detectable efflux of acid under spontaneous (or basal) conditions, this is not significantly different from zero. However, a significant cellular efflux of

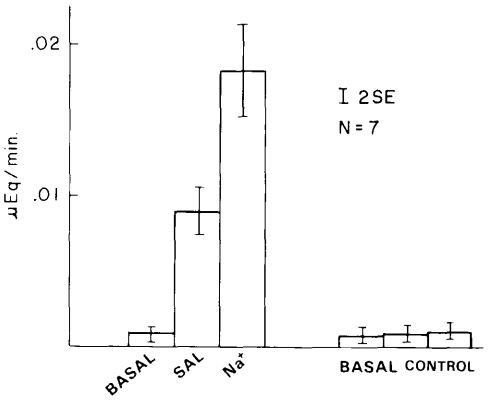

FIG. 2. Titratable H^+ efflux from acid-loaded surface cells resuspended in choline chloride solution (without Hepes; pH 7.0). Basal (or spontaneous) H^+ efflux was measured by pH stat titration using KOH, 0.01 *N*, for 10–15 min. Choline salicylate (SAL), 5 m*M* (pretitrated to pH 7.0), was added and titration was continued for an additional 10–15 min. Subsequently, Na^+, 50 m*M* (pretitrated to pH 7.0), was added as titration was continued for another 10–15 min. (A greater efflux of H^+ was also observed when the sequence of addition was reversed by adding choline salicylate after Na^+.) The "basal control" indicates that if nothing is added to paired cells, the rate of H^+ efflux does not change significantly during measurements made over comparable time intervals.

H^+ occurs following the addition of salicylate. The latter effect is consistent with that observed using AO as a marker of the change in pH_i. These data indicate that salicylate dissipates the pH_i by causing cellular extrusion of protons, but they do not exclude a possible effect of salicylate on spontaneous Cl^-/HCO_3^- exchange. If surface cells secrete HCO_3^- *de novo* through a Cl^-/HCO_3^- exchanger, salicylate conceivably could increase H^+ dissipation by blocking the exchanger and increasing the concentration of intracellular HCO_3^-. Although we have not previously observed evidence of spontaneous anion exchange (14), additional studies were done with SITS, 10^{-7} and 10^{-5} *M* ($N = 5$ for each concentration) added to cells suspended in choline HCO_3^-. Aliquots of cells were taken immediately before and 5, 10, and 20 min after the addition of SITS. These experiments show that the anion-exchange inhibitor SITS has no significant effect on the spontaneous dissipation of the pH_i (data not shown; also see Fig. 8). In a prior study it was shown that HCO_3^--evoked dissipation of the pH_i is inhibited by the same concentrations of SITS (14).

In the absence of salicylate, addition of 50 m*M* Na^+ to the suspension medium of acid-loaded surface cells significantly increases the dissipation of the pH_i (Fig. 3). Sodium-evoked dissipation of the pH_i also occurs in the presence of 2.5, 5.0, and 10.0 m*M* choline salicylate (Fig. 3). The titration data shown in Fig. 2 also confirm that Na^+/H^+ exchange is intact in the presence of salicylate. The increase in titratable H^+ efflux observed for cells exposed to salicylate in choline chloride is increased even more following the addition of Na^+.

Dissipation of the pH_i evoked by Na^+ appears to be greater as the concentration of salicylate increases and is even complete when the concentration of the drug is 10 m*M*, Fig. 3. However, the responses to Na^+ shown in Figs. 2 and 3 do not distinguish between salicylate, causing an increase in the amiloride sensitive (9) and possible amiloride-insensitive Na^+-evoked component of H^+ efflux or merely causing an additive effect independent of Na^+. The latter might occur as a result of the ability of salicylate to dissipate the pH_i in the absence of Na^+ as shown in Fig. 1. Additional experiments were done to clarify the effect of salicylate on the Na^+-evoked efflux of H^+.

The effects of amiloride on Na^+-evoked dissipation of the pH_i in the absence and presence of salicylate are shown in Fig. 4. The enhanced dissipation of the pH_i caused by 50 m*M* Na^+ in the absence of salicylate is blocked by the presence of amiloride, an effect we have reported previously (9). The even greater Na^+-evoked dissipation of the pH_i in the presence of salicylate, however, is only blunted when the cells are also exposed to amiloride. The mean difference in response to Na^+ in the absence and presence of amiloride is not significant between the control and salicylate-treated cells until 20 min ($P < 0.05$). This indicates that the amiloride-sensitive Na^+ pathway is intact. While the similarity in response to Na^+ suggests that salicylate may not increase Na^+/H^+ exchange, additional experiments (done in the same manner as in Fig. 2) involving titration of H^+ cellular efflux using the same concentrations of salicylate and Na^+ show complete inhibition of the effect of Na^+ by amiloride, 1 m*M* ($P < 0.001$, $N = 5$).

Isotopic Na^+ studies were done in order to

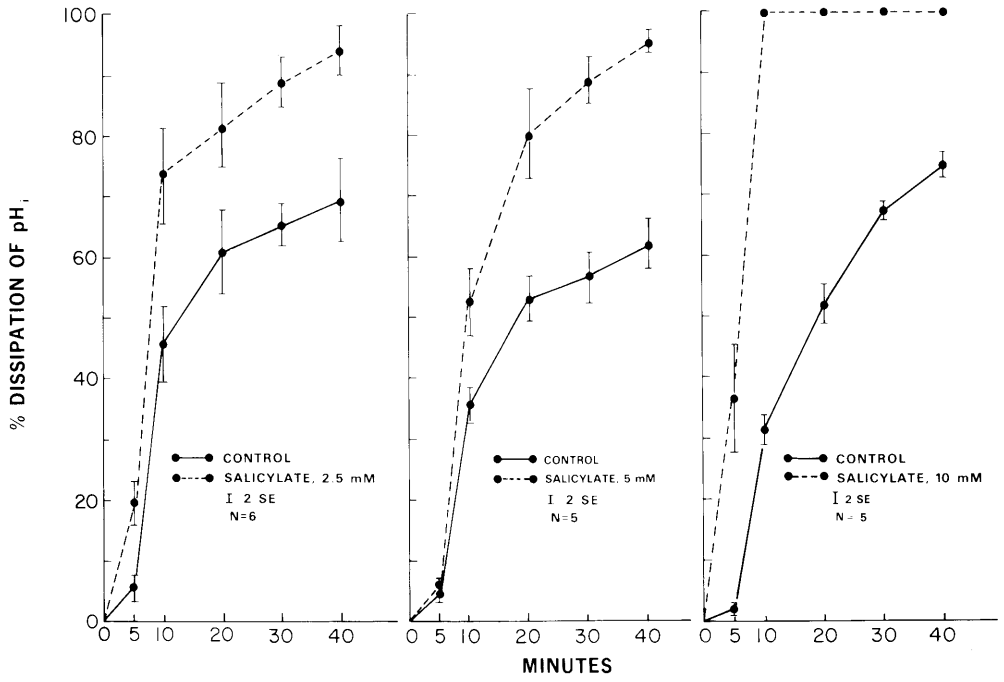

FIG. 3. Effect of Na^+ and choline salicylate on dissipation of pH_i of acid-loaded surface cells. The cells were resuspended in choline chloride solution (pH 7.4). Only Na^+, 50 m*M*, was added to the paired control cells (Control) at 0 time and aliquots were withdrawn at the indicated times. The indicated concentrations of choline salicylate and Na^+ (Salicylate) 50 m*M*, were added at 0 time and aliquots were taken as for the paired control cells. The percentage dissipation of the pH_i was calculated as described under Methods.

further clarify the effect of amiloride on the Na^+-evoked dissipation of the pH_i in the presence of salicylate. Whereas the effects of amiloride using AO and by titration were determined using a near maximal stimulus of Na^+ (50 m*M*) (9), the ^{22}Na-uptake experiments were done using only 1 m*M* Na^+ (Fig. 5). ^{22}Na uptake was determined at 5 min and the counts were normalized to milligrams of cellular protein. Salicylate significantly increases Na^+ uptake compared to control cells and amiloride blocks Na^+ uptake to the same extent in both control and salicylate-treated cells. However, salicylate also increases the amiloride-sensitive component of Na^+ uptake, which is represented by the difference in Na^+ uptake between the non- and amiloride-treated cells.

It is possible that a generalized increase in cellular permeability could account for the effects of salicylate. If cellular permeability is increased by salicylate under the present experimental conditions, one would expect the addition of HCO_3^- to cause more rapid and greater dissipation of the pH_i (14). The effects of choline HCO_3^- are shown in Fig. 6. In the absence of salicylate (and Na^+), HCO_3^- evokes a significant dissipation of the pH_i. Salicylate alone causes dissipation of the pH_i that is almost identical to that observed for HCO_3^- alone. Dissipation of the pH_i is even greater in the combined presence of salicylate and HCO_3^-. However, the difference in pH_i between the control cells and those exposed to HCO_3^- only is not significant at all time points from the difference in pH_i between the cells exposed to salicylate alone and those exposed to salicylate and HCO_3^-. Thus, the combined effect of salicylate and HCO_3^- is only additive. This would not be expected if salicylate increased permeability in general.

The observation that the effect of HCO_3^- on pH_i is not significantly enhanced in the presence of salicylate was made using a HCO_3^- concentration of 20 m*M*. However, a different effect is apparent using lower concentrations of choline HCO_3^- (Fig. 7). In the absence of

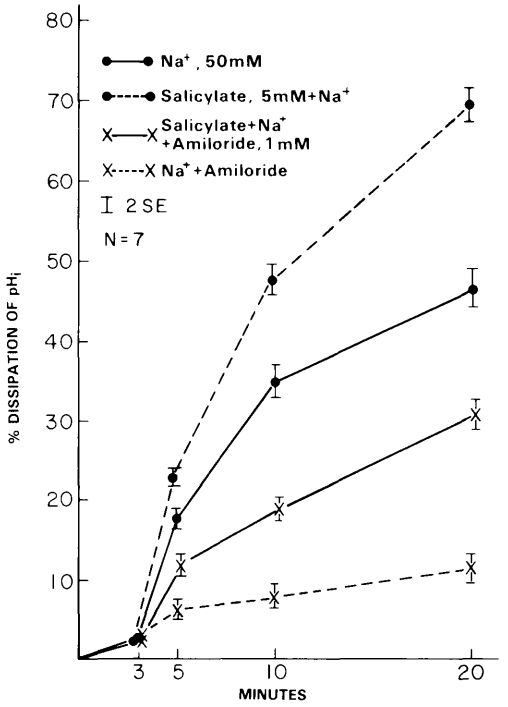

FIG. 4. Effects of amiloride on Na^+ evoked dissipation of pH_i of acid-loaded surface cells in the absence and presence of choline salicylate. All groups of paired cells were resuspended in choline chloride solution (pH 7.4). Amiloride (×) blocks the effect of Na^+ (●). [Previous studies show that this block is complete (9).] The enhanced effect of salicylate on Na^+-evoked dissipation of the pH_i (● - - ●) is partially blocked by amiloride (×—×). The mean difference in response to Na^+ in the absence and presence of amiloride is not significant between the control (●—● minus × - - ×) and salicylate (● - - ● minus ×—×)-treated cells until 20 min ($P < 0.05$). All agents were added at 0 time. Aliquots of the cell suspension were withdrawn at the indicated times and the percentage dissipation of the pH_i was calculated as described under Methods.

salicylate (and Na^+), 5 m*M* HCO_3^- evokes a significant dissipation of the pH_i ($P < 0.01$). Salicylate alone causes dissipation of the pH_i that is not significantly different from that caused by HCO_3^- alone. Dissipation of the pH_i (as also observed for the higher concentration of HCO_3^-) is significantly greater in the combined presence of salicylate and HCO_3^- ($P < 0.01$). However, in contrast to the observation made with the higher concentration of 20 m*M* HCO_3^-, the difference in pH_i between the control cells and those exposed to 5 m*M* HCO_3^- only is significantly *greater* ($P < 0.01$) than the difference in pH_i between the cells exposed to salicylate alone and those exposed to salicylate and 5 m*M* HCO_3^-. This indicates that salicylate blocks the cellular entry of HCO_3^- when a lower concentration of HCO_3^- is present in the suspension medium. The blocking effect of salicylate is barely detectable at an intermediate concentration of HCO_3^-, 10 m*M* ($P = 0.05$, $N = 6$) (data not shown).

Thus, salicylate does not significantly influence HCO_3^--evoked dissipation of the pH_i in the presence of a high concentration of HCO_3^-. However, a blocking effect of salicylate is just noticeable at an intermediate concentration of HCO_3^- and a very apparent blocking effect of salicylate occurs at a low concentration of HCO_3^- in the cellular suspension medium.

The effect of the anion-exchange blocker SITS is shown in Fig. 8. SITS alone is without effect on cells resuspended in choline chloride and inhibits HCO_3^--evoked dissipation of the pH_i. SITS also blocks the effect of salicylate alone on dissipation of the pH_i. Virtually identical effects were observed with DIDS (data not shown).

Discussion. Salicylate increases the dissipation of an intracellular proton gradient es-

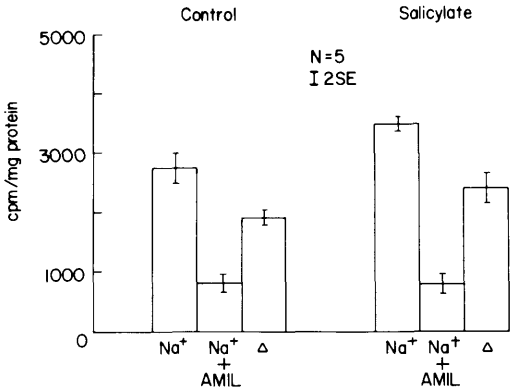

FIG. 5. ^{22}Na uptake of acid-loaded surface cells. The cells were resuspended in choline chloride solution (pH 7.4) containing ouabain, 2 m*M*. Na^+, 1 m*M*, with ^{22}Na was added to each paired cell group. The amiloride (AMIL)-treated cells were preincubated for 1 min with amiloride, 500 μM, prior to the addition of Na^+. The salicylate-treated cells were preincubated for 1 min with choline salicylate, 5 m*M*, prior to the addition of Na^+ or amiloride. Delta (Δ) is the difference between the Na^+ and Na^+ with amiloride values and is a measure of the amiloride-sensitive ^{22}Na uptake. The delta for the salicylate treated cells is significantly greater than that observed for the control cells ($P < 0.01$).

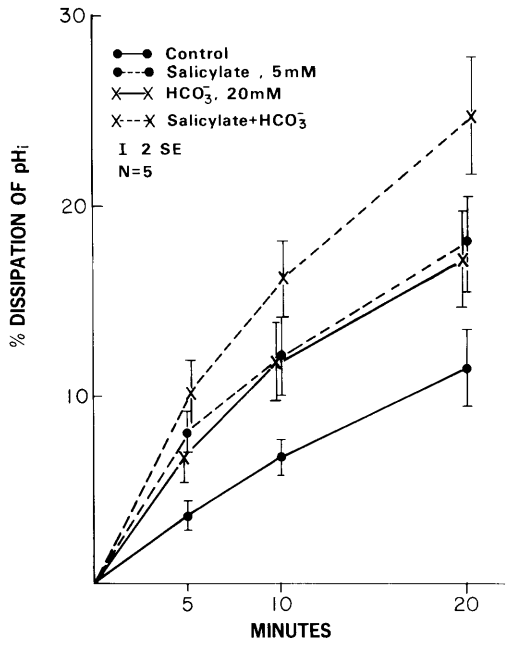

FIG. 6. Effect of choline HCO_3^- on pH_i of acid-loaded surface cells in the absence and presence of salicylate. The paired cells were resuspended in choline chloride solution (pH 7.4). HCO_3^-, 20 m*M*, and/or choline salicylate, 5 m*M*, were added at time 0. Aliquots of the cell suspension were withdrawn at the indicated times and the percentage dissipation of the pH_i was calculated as described under Methods. Only choline chloride was added to the control cells.

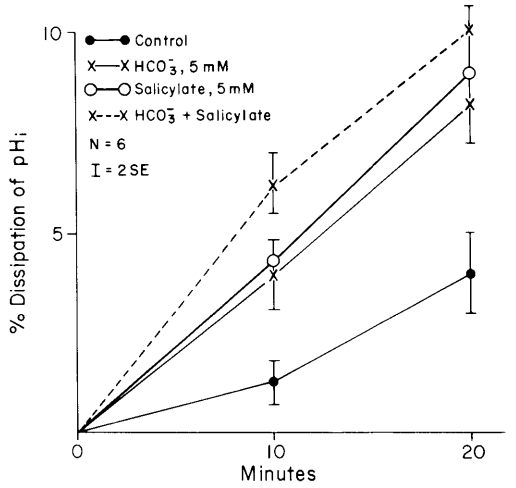

FIG. 7. Effect of a lower concentration of choline HCO_3^-, 5 m*M*, on pH_i of acid-loaded surface cells in the absence and presence of choline salicylate, 5 m*M*. The experimental protocol is the same as described in the legend of Fig. 6.

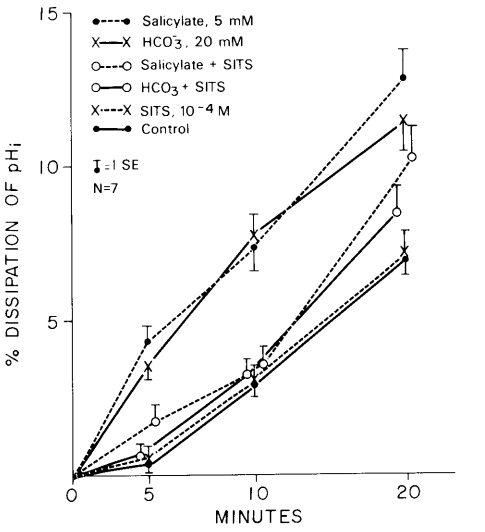

FIG. 8. Effect of SITS on salicylate and HCO_3^- evoked dissipation of the pH_i. The cells were resuspended in choline chloride solution (pH 7.4). HCO_3^-, salicylate, and/or SITS were added at time 0. Aliquots of cells were taken at the indicated times and the percentage dissipation of the pH_i was calculated as described under Methods. Control cells were resuspended in only choline chloride solution.

tablished in isolated gastric mucosal surface cells suspended in an alkaline medium. Since cellular dissolution was not observed under the present experimental conditions as well as in a prior study (15), it appears that dissipation of the gradient is a result of H^+ efflux. The latter could predominantly involve one or more mechanisms. For example, this process may be a result of stimulation of a H^+ exchange phenomenon or passive H^+ diffusion from a cell with increased permeability or proton capture by salicylate as it enters the cell. Alternatively, changes in cellular production and/or secretion of HCO_3^- also could influence the intracellular pH. The data indicate that there is involvement of more than one of these mechanisms.

The Na^+/H^+ exchange mechanism of surface cells not only remains intact but also appears to be enhanced upon exposure to salicylate. This is indicated not only by the changes in fluorescence of acridine orange but also by the increase in isotopic Na^+ influx and titratable H^+ efflux. The ability of salicylate to facilitate cation exchange is related to an increase in amiloride-sensitive Na^+ entry into the surface cells. A previous study demonstrated that

Na^+-evoked dissipation of an intracellular proton gradient is not affected by ouabain (9). Thus, a conductive Na^+/H^+ exchange mechanism probably is not involved in the increased cellular entry of Na^+ as a result of exposure to salicylate.

While salicylate increases Na^+/H^+ exchange via a pathway predominantly sensitive to amiloride, the data also suggest that an additional mechanism is involved in the dissipation of the intracellular proton gradient. Titration of a Na^+-free suspension medium containing acid-loaded cells shows that H^+ is extruded from the cell in the presence of salicylate. Thus, salicylate also causes a proton leak independent of extracellular Na^+. Salicylate possibly shuttles protons across the cell membrane. Although salicylate anion is less permeable than the undissociated form of the drug, an intracellular accumulation does occur at neutral pH (5, 16). Once in the cell, salicylate may combine with H^+, making the drug more lipid permeable and facilitate the removal of H^+.

Another mechanism might involve an alteration of intracellular HCO_3^- production or secretion by salicylate. Gastric mucosal surface cells are believed to secrete HCO_3^-, but studies suggesting this involve indirect evidence. It is unlikely that choline salicylate alters intracellular pH in the absence of Na^+ by affecting extrusion or secretion of HCO_3^-, since an inhibitor of anion exchange (SITS) was without effect on the intracellular proton gradient under the present experimental conditions.

Salicylate is ordinarily thought of as causing a generalized increase in gastric mucosal permeability, but this is only true in the presence of luminal acid (17). In the presence of intracellular and absence of extracellular acid, however, salicylate does not cause a generalized increase in ionic conductance of surface cells. While salicylate increases Na^+ permeability of surface cells, this appears to be selective and not a result of a generalized increase in cellular conductance to both cation and anion. If the latter were the case, one would expect an effect of HCO_3^- on the intracellular proton gradient to exceed that observed in the absence of salicylate. Salicylate does not alter the effect of a relatively high concentration of HCO_3^- (20 m*M*) on the intracellular proton gradient. This is in contrast to lower concentrations of HCO_3^- (10 and 5 m*M*), in which case salicylate impairs HCO_3^--evoked dissipation of the gradient. It is likely that the decrease in anion permeability observed at the lower concentration of HCO_3^- is masked by the gradient effect of the higher concentration of HCO_3^-. Thus, salicylate appears to have a selective effect on (increasing) cation and (decreasing) anion permeability. Ion selective effects of salicylate have also been observed using intact antral mucosa bathed in a neutral pH solution (17). The ability of SITS to partially block salicylate-induced dissipation of the pH_i suggests that salicylate enters the cell via an anion/Cl^- exchange. Thus, the ability of salicylate to decrease HCO_3^- entry into the cell may involve competition for the Cl^-/HCO_3^- exchange site in addition to a less specific negative charge effect (18, 19).

In summary, salicylate dissipates an intracellular proton gradient by two mechanisms. One affects amiloride-sensitive Na^+/H^+ exchange whereas the other enhances a leak of H^+ independent of extracellular Na^+. The latter, however, is not due to a generalized increased in cellular permeability.

1. Davenport HW. Gastric mucosal injury by fatty and acetylsalicylic acids. Gastroenterology **46**:245–253, 1964.
2. Davenport HW. Damage to the gastric mucosa: Effects of salicylate and stimulation. Gastroenterology **49**: 189–196, 1965.
3. Davenport HW. Potassium fluxes across the resting and stimulated gastric mucosa: Injury by salicylate and acetic acids. Gastroenterology **49**:238–245, 1965.
4. Davenport HW. Fluid produced by by the gastric mucosa during damage by acetic and salicylic acids. Gastroenterology **50**:487–499, 1966.
5. Davenport HW. Gastric mucosal hemorrhage in dogs: Effects of acid, aspirin and alcohol. Gastroenterology **56**:439–449, 1969.
6. Fromm D, Schwartz JH, Quijano R. The effects of salicylate and bile salt on ion transport by isolated gastric mucosa of the rabbit. Amer J Physiol **230**:391–326, 1976.
7. Spenney JG, Bhown M. Effect of acetylsalicylic acid on the amphibian gastric mucosa. I. Electrophysiological and permeability changes. Gastroenterology **73**: 785–789, 1977.
8. Hingson DJ, Ito S. Effect of aspirin and related compounds on the fine structure of mouse gastric mucosa. Gastroenterology **61**:156–177, 1971.
9. Olender EJ, Fromm D, Furukawa T, *et al.* H^+ disposal by rabbit gastric mucosal surface cells. Gastroenterology **86**:698–705, 1984.

10. Tanaka K, Fromm D, Hill RB, *et al.* Isolation and viability of gastric mucosal surface cells of the rabbit. J Surg Res **33**:265–279, 1982.
11. Lee HC, Quintanilha AT, Forte JG. Energized gastric microsomal membrane vesicles: An index using metachromatic dyes. Biochem Biophys Res Commun **72**:1179–1186, 1976.
12. Lee HC, Forte JG. A study of H^+ transport in gastric microsomal vesicles using fluorescent probes. Biochim Biophys Acta **508**:339–356, 1978.
13. Lowry OH, Rosebrough NJ, Farr AL, *et al.* Protein measurement with the Folin phenol reagent. J Biol Chem **193**:265–275, 1951.
14. Furukawa T, Olender E, Fromm D, *et al.* The effects of cyclic adenosine monophosphate and prostaglandins on Na^+- and HCO_3^--induced dissipation of a proton gradient in isolated gastric mucosal surface cells of rabbits. Gastroenterology **89**:500–506, 1985.
15. Tanaka K, Fromm D. Effects of bile acid and salicylate on surface and glandular cells of rabbit stomach. Surgery **93**:660–663, 1983.
16. Spenney JG, Marshall G. Uptake and intracellular concentration of salicylate and *p*-hydroxybenzoate in in vitro fundic gastric mucosa. Gastroenterology **84**: 1318, 1983.
17. Fromm D. Ion selective effects of salicylate on antral mucosa. Gastroenterology **71**:743–749, 1976.
18. Weith JO. Effect of some monovalent anions on chloride and sulphate permeability of human red cells. J. Physiol **207**:581–569, 1970.
19. Barker JL, Levitan H. Salicylate: Effect on membrane permeability of molluscan neurons. Science **172**:1245–1247, 1971.

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