NH₄Cl inhibition of acid secretion: possible involvement of an apical K⁺ channel in bullfrog oxyntic cells

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NH₄Cl inhibition of acid secretion: possible involvement of an apical K⁺ channel in bullfrog oxyntic cells. Am J Physiol Gastrointest Liver Physiol 279: G400–G410, 2000.—This study was undertaken to determine the mechanism by which ammonium chloride (NH₄Cl) inhibits stimulated acid secretion in the bullfrog gastric mucosa. To this end, four possible pathways of inhibition were studied: 1) blockade of basolateral K⁺ channel, 2) blockade of ion transport activity, 3) neutralization of secreted H⁺ in the luminal solution, or 4) ATP depletion. Addition of nutrient 10 mM NH₄Cl (calculated NH₃ concentration = 92.5 μM and NH₄⁺ concentration = 9.91 mM) inhibited acid secretion within 30 min. Inhibition of acid secretion did not occur by blockade of basolateral K⁺ channel activity or ion transport activity or by neutralization of the luminal solution. Although ATP depletion occurred in the presence of NH₄Cl, the magnitude of ATP depletion in 30 min was not sufficient to inhibit stimulated acid secretion. By comparing the effect of NH₄Cl on the resistance of inhibited or stimulated tissues, we demonstrate that NH₄Cl acts specifically on stimulated tissues. We propose that NH₄Cl blocks activity of an apical K⁺ channel present in stimulated oxyntic cells. Our data suggest that the activity of this channel is important for the regulation of acid secretion in bullfrog oxyntic cells.

Rana catesbeiana; gastric mucosa; ammonia

HYPОCHLOORHYDRIA AND INFLAMMATION are hallmarks of early infection with Helicobacter pylori (HP), a pathogenic bacterium that colonizes the gastric antrum and fundus and leads to chronic-active gastritis and peptic ulcer disease. During early infection with HP, it has been suggested that acid secretion may be suppressed by one or more products of HP or by inflammatory mediators that act directly on parietal cells (3, 21). Among the various factors liberated by HP (3), urease may be the most important because it leads to the production of large quantities of ammonia (NH₃) that can affect cell function and viability (23, 30, 36, 37, 40). Hypochlorhydria can also occur during chronic renal or hepatic failure or after renal transplantation, when circulating levels of blood NH₃ are extremely high (9, 16, 24).

NH₃ is a small molecule of 17 Da that acts as a weak base and is at equilibrium with its protonated form (NH₄⁺) at a pKₐ of 9.233. Thus most of the NH₃ produced by HP exists as NH₄⁺ in the acidic environment of the gastric lumen. It has been shown (2) that the apical (or luminal) surface of both gastric parietal and chief cells is relatively impermeable to NH₃ and NH₄⁺, suggesting that NH₃ formed in the gastric lumen during HP infection would be unable to alter gastric epithelial cell function. However, Yanaka et al. (40) showed that luminal administration of 115 mM ammonium chloride (NH₄Cl) at pH 8 (2.7 mM NH₃) resulted in a significant decrement in tissue resistance (R) and an increase in H⁺ back-diffusion that did not occur when the same concentration of NH₄Cl was used at pH 5 (3 μM NH₃), indicating that luminal NH₃ can cause an increase in the paracellular permeability of the gastric mucosa. In addition, it was recently shown (26, 41) that the vacuolating cytotoxin produced in VacA+ HP bacteria increases the paracellular permeability of cultured gastric (GSM06), colonic (T84), and kidney (Madin-Darby canine kidney) cells. Thus both NH₃ and vacuolating cytotoxin may facilitate the movement of gastric luminal contents including NH₃ to the serosal compartment during infection.

Whereas the apical surface of gastric epithelial cells is impermeable to NH₃, NH₃ is freely permeable across the basolateral cell membrane and NH₄⁺ can enter cells via cation channels, pumps, or exchange proteins (2). Basolateral exposure of the bullfrog gastric mucosa to 30 mM NH₄Cl at pH 7.2 (0.47 mM NH₃ and 29.53 mM NH₄⁺) rapidly and completely inhibits acid secretion and accelerates H⁺ back-diffusion while it alkalinizes, induces depolarization, and decreases electrogenic Cl⁻ secretion in bullfrog oxynticopeptic cells (40).

Basolateral exposure of gastric epithelial cells to NH₃/NH₄⁺ also allows these substances to interact directly with basolateral ion channels, transporters, and exchangers. Although little is known about the effects of NH₃ and/or NH₄⁺ on basolateral ion transporters/exchangers in the gastric mucosa, it was suggested by Yanaka et al. (40) that NH₄Cl may block basolateral K⁺ channel activity. This conclusion was derived from the observation that high K⁺ (15 mM) in the nutrient solution attenuated the decrease in both potential difference (PD) and R that occurred after exposure to 30

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To stimulate acid secretion, 0.1 mM histamine and 1 mM carbachol were added to the nutrient solution and maximal acid secretion was accomplished within 90 min. After maximal stimulation, one of the following substances was added to the nutrient solution: 1) NH₄Cl (1–10 mM), 2) methylamine (50 mM), 3) imidazole (200 μM and 10 mM), or 4) potassium cyanide (KCN, 250 μM–2 mM). Methylamine is a primary amine weak base just slightly larger and less permeable than NH₃. Methylamine has been shown to enter cells and block K⁺-channel activity in intestinal T84 cells (14, 17, 27). Imidazole is a nonamine weak base that alkalizes the cytoplasm and partitions into acidic intracellular compartments, as does NH₃. KCN inhibits mitochondrial respiration and the production of ATP. All solutions were added from concentrated stock, and then the pH was adjusted to 7.1 (imidazole) or 7.2 (NH₄Cl, methylamine, or KCN). After 30 min, tissues were washed, and acid secretion was measured in the presence of histamine plus carbachol for an additional 90 min. In one set of experiments, tissues were stimulated for 90 min with 20 mM forskolin (in the absence of histamine and carbachol) and then incubated for 30 min with 10 mM NH₄Cl to verify that NH₄Cl inhibits stimulated acid secretion distal to the H₂ receptor binding and the production of cAMP. Inhibition of acid secretion with H₂ or proton-pump inhibitors was accomplished by washing both luminal and nutrient compartments with buffer and then incubating the tissues with 1 mM aqueous cimetidine or 0.3 mM omeprazole in 0.1% DMSO. Histamine, carbachol, imidazole, methylamine, potassium cyanide, and cimetidine were purchased from Sigma Chemical (St. Louis, MO). Forskolin was purchased from CalBiochem (San Diego, CA). Omeprazole was a kind gift from Astra Merck.

To block basolateral K⁺-channel activity, tissues stimulated with histamine plus carbachol for 90 min were incubated with one of four K⁺-channel blockers: 1) Ba²⁺ (0.5, 1, and 5 mM); 2) tetraethylammonium (TEA; 1, 5, and 10 mM); 3) clotrimazole (10 μM); or 4) tolbutamide (10 and 100 μM). Ba²⁺ and TEA are wide-spectrum K⁺-channel blockers of which Ba²⁺ is known to inhibit K⁺-channel activity in frog oxyntic cells (20). Clotrimazole, and imidazole antibiotic that blocks cAMP and Ca²⁺-activated K⁺-channel activity in T84 cells (31, 32), was tested for its ability to inhibit cAMP and Ca²⁺-activated K⁺ channels present in frog oxyntic cells. Tolbutamide, a sulfonyleurea compound that blocks ATP-dependent K⁺-channel activity in excitable tissues, was also tested for its ability to block K⁺-channel activity in frog oxyntic cells. All K⁺-channel blockers were added to the nutrient solution from concentrated aqueous stock solutions or in DMSO where the concentration of DMSO did not exceed 0.1%. Acid secretion and PD were measured for 3 h in the presence of each K⁺-channel blocker. Ba²⁺, TEA, clotrimazole, and tolbutamide were purchased from Sigma Chemical.

Acid secretion was measured using a Radiometer pH-stat device and calculated from the volume of 10 mM NaOH needed to titrate the mucosal solution to a constant pH of 4.7. Transmucosal PD was continuously monitored using KCl-saturated agar bridges connected via two calomel electrodes to a voltmeter. R was calculated from Ohm’s law.

Electron microscopy. Frog tissues from the Ussing chamber were fixed overnight at 4°C with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), postfixed for 1 h at 4°C with 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4), and stained overnight at 4°C with 2% aqueous uranyl acetate. Tissues were dehydrated in graded alcohols and propylene oxide and embedded in LX112 resin. Thin sections, cut parallel to the long axis of gastric glands, were placed on Form-
var- and carbon-coated grids and examined with a JEOL 100CX electron microscope.

NH₃ and methylamine flux studies. NH₃ flux from nutrient to luminal solutions was measured enzymatically by the reductive amination of 2-oxoglutarate to glutamate and reduction of nicotinamide adenine dinucleotide phosphate (NADPH) to NADP in the presence of glutamate dehydrogenase and NH₃. In brief, 0.1 ml of nutrient or luminal solution was mixed with 1.0 ml of NH₃ assay solution containing 3.4 mM 2-oxoglutarate and 0.23 mM NADPH in 30 mM Tris buffer (pH 8.0), and the optical density (OD) of the solution was determined at a wavelength of 340 nm (initial OD). After the addition of 0.008 ml of L-glutamate dehydrogenase to each sample, the OD at 340 nm was determined (final OD). The final OD₃₄₀ was subtracted from the initial OD₃₄₀, and the concentration of NH₃ (not NH₄⁺) was determined from a standard curve. Oxoglutarate, NADPH, and glutamate dehydrogenase were purchased from Sigma Diagnostics (St. Louis, MO).

For flux studies with NH₄Cl, frog gastric mucosae were mounted in Ussing chambers and then stimulated with histamine plus carbachol for 90 min. NH₄Cl (10 mM) was added to the nutrient solution, and the nutrient and luminal solutions were assayed for NH₃ to establish starting conditions. After 30 min in nutrient 10 mM NH₄Cl, samples of both nutrient and luminal solutions were collected and the concentration of NH₃ was determined, as described above.

For flux studies with methylamine, frog gastric mucosae were mounted in Ussing chambers and one of the paired tissues was stimulated for 90 min with histamine plus carbachol while the other was inhibited with cimetidine (as described above). Methylamine (50 mM), containing 5 μCi of [¹⁴C]methylamine, was added to the nutrient solution, and duplicate 0.5-ml samples were taken from the luminal solution every 15 min for 1 h. Samples were mixed with Atom-light scintillation fluid (Packard Instruments, Meriden, CT), and the dpm were counted in a Packard scintillation counter. The concentration of methylamine in the luminal solution was calculated by established techniques.

ATP assay. Isolated gastric glands were prepared from the rabbit fundus after high-pressure retrograde aortic perfusion by the technique previously described in detail by Carter et al. (4) as modified from Berglindh and Obrink (1). Isolated glands were maintained in the resting state by the addition of 10 μM cimetidine. Intracellular ATP was measured by the luciferin/luciferase assay in resting glands that were incubated for 30 min at 37°C in the presence or absence of 10 mM NH₄Cl (pH 7.4) or 2 mM KCN. ATP was extracted from the glands after precipitation of proteins with 2% TCA and 2 mM EDTA as described previously (38). Bioluminescence was measured in an LKB Wallac model 1250 luminometer (Turku, Finland), and the concentration of ATP was determined with an ATP standard. Results were expressed as micromoles of ATP per milligram of protein, which was determined using the BioRad protein assay.

Statistical analysis. Combined data were expressed as means ± SE. Statistical analyses of data were done with SigmaStat software (Jandel Scientific Software, San Rafael, CA) using the unpaired t-test for analysis of two groups or one-way ANOVA for many groups. Differences were regarded as statistically significant at \( P < 0.05 \).

**RESULTS**

Nutrient NH₄Cl rapidly and reversibly inhibits stimulated acid secretion. Acid secretion in frog fundic mucosa attained a maximal rate of $5.80 ± 0.12 \mu\text{eq} \cdot h^{-1} \cdot \text{cm}^{-2}$ by 90 min after stimulation (Fig. 1A). While \( R \) remained constant at $164.7 ± 5.61 \Omega \cdot \text{cm}^2$ (Fig. 1B), PD declined slowly to $6.00 ± 0.70 \text{mV}$ by 90 min after stimulation (Fig. 1C). Similar results were obtained after stimulation with forskolin (data not shown).

When 10 mM NH₄Cl was added to the nutrient solution (pH 7.2) of tissues stimulated with either

![Fig. 1. Effect of basolateral NH₄Cl on stimulated acid secretion and electrophysiology in the bullfrog gastric mucosa. In tissues stimulated with histamine plus carbachol for 90 min, addition of 10 mM NH₄Cl to the nutrient buffer completely abolished acid secretion and resulted in an increase in both resistance (\( R \)) and potential difference (PD) within 30 min. Removal of NH₄Cl was followed by a complete recovery of acid secretion (\( A \)), R (\( B \)), and PD (\( C \)) within 30 min. Data were obtained from 21 experiments and are expressed as means ± SE.](http://ajpgi.physiology.org/content/180/2/402/F1)

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histamine plus carbachol (Fig. 1A) or forskolin (data not shown), the rate of acid secretion declined to $0.60 \mu$eq $\cdot$ h$^{-1}$ $\cdot$ cm$^{-2}$ within 30 min. Although incubation of stimulated mucosa with 1–9 mM NH$_4$Cl resulted in an inhibition of acid secretion (data not shown), 10 mM NH$_4$Cl was the lowest concentration required to result in complete inhibition of acid secretion within 30 min. Concomitant with a rapid reduction in acid secretion in the presence of 10 mM NH$_4$Cl, $R$ and PD increased significantly to $306.5 \pm 16.11 \Omega \cdot$ cm$^2$ and $28.48 \pm 1.47$ mV, respectively. Even though acid secretion was completely inhibited in 30 min by 10 mM NH$_4$Cl, tissue morphology at this time point showed oxynticopeptic cells in a stimulated rather than inhibited configuration (Fig. 2). The rate at which acid secretion declined over time in the presence of 10 mM NH$_4$Cl was 3.6 times faster than with cimetidine or 3.2 times faster than with omeprazole (Table 1).

When 10 mM NH$_4$Cl was washed from the nutrient bath and tissues incubated in buffer without NH$_4$Cl, acid secretion, $R$, and PD returned to near-normal values of $5.51 \pm 0.16 \mu$eq $\cdot$ h$^{-1}$ $\cdot$ cm$^{-2}$, $159.0 \pm 16.83 \Omega \cdot$ cm$^2$, and $7.95 \pm 2.65$ mV, respectively, within 90 min (Fig. 1).

Acid secretion is rapidly inhibited by methylamine but not by imidazole. Methylamine and imidazole were used to determine whether other small primary amines or weak bases, respectively, inhibit acid secretion (Fig. 3). Methylamine inhibited stimulated acid secretion from $5.81 \pm 0.125$ to $0.081 \pm 0.056 \mu$eq $\cdot$ h$^{-1}$ $\cdot$ cm$^{-2}$ within 30 min at a minimum concentration of 50 mM (Fig. 3A). However, when methylamine was washed from the nutrient solution, acid secretion returned to only 60% ($3.74 \pm 0.41 \mu$eq $\cdot$ h$^{-1}$ $\cdot$ cm$^{-2}$) of the starting level within 60 min (Fig. 3A). When imidazole was used at a concentration similar to that of NH$_3$ in the nutrient buffer ($100 \mu$M imidazole vs. $92.5 \mu$M NH$_3$), acid secretion decreased slowly from $5.47 \pm 0.26$ to $4.86 \pm 0.29 \mu$eq $\cdot$ h$^{-1}$ $\cdot$ cm$^{-2}$ in 3 h (180 min). This reduction in acid secretion over time was not signifi-

Fig. 2. Electron micrograph of a gastric gland from tissues stimulated with histamine plus carbachol for 90 min and then incubated for 30 min with 10 mM NH$_4$Cl. Note that oxynticopeptic cells (OP) lining the gastric gland have an apical secretory surface in the stimulated configuration (arrowheads) with many surface folds projecting into the gland lumen (L). LP, lamina propria. Original magnification, $\times 4,600$; bar, $5 \mu$m.
The rate of methylamine flux was \(0.601 \pm 0.114\) and \(2.25 \pm 0.088 \mu \text{M} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}\) in inhibited and stimulated tissues, respectively (Fig. 4). The rate of acid secretion was \(0.099 \pm 0.089\) and \(7.0 \pm 0.3 \mu \text{eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}\) in inhibited and stimulated tissues, respectively. As it would require \(7.0 \mu \text{M} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}\) of methylamine in the luminal solution to titrate all of the H\(^+\) generated during acid secretion, our data demonstrate that titration alone cannot account for the total inhibition of acid secretion that occurs with methylamine. Rather, acid secretion would decline slowly over time, a situation similar to results with other weak bases such as imidazole.

Blockade of basolateral K\(^+\)-channel activity does not mitigate the inhibitory effects of NH\(_4\)Cl on stimulated acid secretion. Ba\(^{2+}\) at 0.5 and 1 mM reduced acid secretion in 3 h (180 min) from 5.23 \pm 0.29 and 5.35 \pm 0.86 \mu \text{Eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}\) in stimulated tissues.}

**Table 1. Rate of acid secretion in the presence of cimetidine, omeprazole, NH\(_4\)Cl, or KCN**

<table>
<thead>
<tr>
<th>Acid Secretion ((\mu \text{eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}))</th>
<th>Time (min)</th>
<th>Correlation Coefficient</th>
<th>Rate of Decline in Acid Secretion ((\mu \text{eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}) (\cdot \text{min}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cimetidine</td>
<td>5.59 \pm 0.13</td>
<td>90</td>
<td>-0.997</td>
</tr>
<tr>
<td></td>
<td>3.51 \pm 0.21</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.83 \pm 0.22</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Omeprazole</td>
<td>5.45 \pm 0.30</td>
<td>90</td>
<td>-0.989</td>
</tr>
<tr>
<td></td>
<td>2.03 \pm 0.21</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.30 \pm 0.10</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>NH(_4)Cl</td>
<td>5.80 \pm 0.12</td>
<td>90</td>
<td>-0.999</td>
</tr>
<tr>
<td></td>
<td>2.73 \pm 0.14</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.38 \pm 0.08</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>KCN</td>
<td>5.63 \pm 0.12</td>
<td>90</td>
<td>-0.998</td>
</tr>
<tr>
<td></td>
<td>3.03 \pm 0.25</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.864 \pm 0.15</td>
<td>110</td>
<td></td>
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</tbody>
</table>

Values are means \(\pm\) SE. Tissues from the frog gastric mucosa were stimulated for 90 min with histamine plus carbachol, washed, and then incubated for 180 min with 1 mM cimetidine or 0.3 mM omeprazole or for 30 min with 10 mM ammonium chloride (NH\(_4\)Cl) or 2 mM potassium cyanide (KCN). The rate at which acid secretion declined over time was determined from the linear portion of the inhibition curve for each compound. Data were obtained from 6–7 experiments (cimetidine and omeprazole), 21 experiments (NH\(_4\)Cl), or 6 experiments (KCN).

siently different from that of control tissues (Fig. 3B). Increasing the concentration of imidazole 50-fold to 10 mM (5 mM imidazole, 5 mM imidazole\(^{2-}\)) decreased acid secretion rapidly from \(6.60 \pm 0.43\) to \(4.60 \pm 0.43 \mu \text{eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}\) in 30 min and to \(4.06 \pm 0.46 \mu \text{eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}\) in 3 h (data not shown). When 10 mM nutrient NH\(_4\)Cl was added to imidazole-treated tissues, acid secretion was inhibited to \(0 \pm 0 \mu \text{eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}\) within 30 min (Fig. 3B).

Serosal-to-mucosal flux of NH\(_3\) and methylamine is not great enough to neutralize secreted H\(^+\). To determine whether nutrient 10 mM NH\(_4\)Cl or 50 mM methylamine blocks acid secretion by moving rapidly from nutrient to luminal solutions, and thus neutralizing secreted H\(^+\), the flux of NH\(_3\) and methylamine was measured.

After addition of 10 mM NH\(_4\)Cl to the nutrient solution, the NH\(_3\) concentration was \(599.76 \mu \text{M}\). At the start, NH\(_3\) was not detectable in the luminal solution. By 30 min after addition of 10 mM NH\(_4\)Cl to the nutrient solution, the nutrient NH\(_3\) concentration was reduced to \(538.3 \pm 6.7 \mu \text{M}\). Thus \(61.46 \mu \text{M} \text{NH}_3\) was lost from the nutrient solution. However, luminal NH\(_3\) concentration after 30 min increased in only one group \(n = 4\), resulting in a mean concentration of \(0.00133 \pm 0.00133 \mu \text{M}\). These results suggest that the NH\(_3\) lost from the nutrient buffer within 30 min resides in the cells and intracellular spaces of the tissue.

It is possible, however, that NH\(_3\) cannot be detected in the luminal solution of stimulated tissues because the forward movement of NH\(_3\) is balanced by the backward movement of NH\(_4\)\(^+\) into the cell by H\(^+\)-K\(^+\)-ATPase, as has been described previously (8). Thus serosal-to-mucosal flux studies with methylamine, a compound that should not interact with H\(^+\)-K\(^+\)-ATPase, were performed (Fig. 4).
0.22 \mu\text{eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-2} \text{ to } 4.12 \pm 0.38 \text{ and } 4.20 \pm 0.21 \mu\text{eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}, \text{ respectively (Fig. 5A). This decline in the rate of acid secretion over time was not significantly different from that of control tissues (Fig. 5A). In contrast, incubation of stimulated tissues with 5 mM Ba}^{2+} \text{ decreased the rate of acid secretion from 5.19 \pm 0.22 \text{ to } 2.89 \pm 0.33 \mu\text{eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-2} \text{ in } 3 \text{ h (180 min). This reduction in the rate of acid secretion was significantly different from that of control tissues (Fig. 5A).}

Acid secretion in the presence of another wide-spectrum K\textsuperscript{+}-channel blocker such as TEA (Fig. 6A), tolbutamide, which blocks ATP-dependent K\textsuperscript{+}-channel activity (Fig. 6B), or clotrimazole, which blocks Ca\textsuperscript{2+}- and cAMP-dependent K\textsuperscript{+}-channel activity (Fig. 6B), showed no significant decline compared with control tissues. When tissues treated with 0.5–5 mM Ba\textsuperscript{2+}, 1–10 mM TEA, 10–100 \mu\text{M tolbutamide, or } 10 \mu\text{M clotrimazole were incubated with 10 mM NH}_4\text{Cl, acid secretion was inhibited to } 0 \mu\text{eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-2} \text{ within } 30 \text{ min in all groups (Figs. 5 and 6).}

Addition of 0.5, 1, or 5 mM Ba\textsuperscript{2+} to the nutrient solution resulted in a significant increase in R in all groups (Fig. 5B). Whereas the largest increase in R occurred in tissues incubated with 5 mM Ba\textsuperscript{2+}, tissues incubated with 0.5 and 1 mM Ba\textsuperscript{2+} also showed a significant increase in R compared with control tissues (Fig. 5B). When tissues were treated with TEA (Fig. 6C), tolbutamide, or clotrimazole (data not shown), the mean R was not significantly different from that of control tissues. Tissues incubated with 0.5 and 1 mM Ba\textsuperscript{2+} (Fig. 5B), TEA (Fig. 6C), tolbutamide, or clotrimazole and 10 mM NH\textsubscript{4}Cl showed an increase in R within 30 min.

Whereas the mean PD of tissues incubated with Ba\textsuperscript{2+} was increased, this increase was not significantly
different from that of control tissues (Fig. 5C). Similar results were obtained for tissues incubated with TEA, tolbutamide, or clotrimazole (data not shown). Tissues incubated with Ba$^{2+}$ (Fig. 5C), TEA, tolbutamide, or clotrimazole (data not shown) and 10 mM NH$_4$Cl showed an increase in PD within 30 min.

Blockade of basolateral transporter activity does not significantly abrogate NH$_3$-induced inhibition of acid secretion. Incubation of stimulated tissues with 100 µM bumetanide, 1 mM amiloride, or 1 mM ouabain for 60 min caused no significant reduction in acid secretion (Figs. 7A). In contrast, acid secretion was reduced from 5.25 ± 0.12 to 2.29 ± 0.18 µeq·h$^{-1}$·cm$^{-2}$ in stimulated tissues incubated with 0.3 mM DIDS for 60 min (Fig. 7B). When stimulated tissues were incubated for 30 min with one of the four ion transport blockers and then further incubated with 10 mM NH$_4$Cl, acid secretion declined to 0.645 ± 0.278, 0.188 ± 0.140, 0, and 0 µeq·h$^{-1}$·cm$^{-2}$ for bumetanide, amiloride, ouabain, and DIDS, respectively, within 30 min (Fig. 7). Similar results were obtained when a combination of bumet-
anide, amiloride, and oubain (followed by NH₄Cl) was added to stimulated tissues (data not shown).

KCN inhibits stimulated acid secretion similarly to 10 mM NH₄Cl but results in significantly greater depletion of intracellular ATP. Acid secretion in the frog gastric mucosa was inhibited in a dose-dependent manner after addition of the metabolic inhibitor potassium cyanide (KCN). Whereas 250 μM KCN significantly reduced acid secretion (data not shown), 2 mM KCN was required to inhibit acid secretion to 0.33 ± 0.04 μeq·h⁻¹·cm⁻² within 30 min (Fig. 8A). The rate of reduction of acid secretion with 2 mM KCN was similar to that of 10 mM NH₄Cl and three times faster than with cimetidine or omeprazole (Table 1). Inhibition of acid secretion with 2 mM KCN was accompanied by an increase in R from 112.4 ± 8.16 to 613.7 ± 38.19 Ω·cm² and PD from 4.72 ± 2.59 to 15.05 ± 1.20 mV (data not shown).

To determine whether 10 mM NH₄Cl reduced intracellular ATP similarly to 2 mM KCN, we prepared gastric glands from the rabbit and measured intracellular ATP. In resting glands, 2 mM KCN significantly reduced intracellular ATP within 30 min to 0.982 ± 0.051 μM/mg protein (21.2% of control) whereas 10 mM NH₄Cl decreased intracellular ATP to 3.76 ± 0.12 μM/mg protein (83% of control, Fig. 8B).

NH₄Cl does not increase R of cimetidine-inhibited tissues. Tissues were incubated with cimetidine until acid secretion reached 0 μeq·h⁻¹·cm⁻²; experiments were performed only if the R of paired tissues were different by 10% or less. When 10 mM NH₄Cl was added to the nutrient solution of cimetidine-inhibited tissues (pH 7.2), there was no change in R compared with control tissues incubated with NaCl (Fig. 9). These results are in contrast to those in tissues stimulated with histamine plus carbachol (Fig. 1B), where tissue R increased significantly in the presence of 10 mM NH₄Cl. In addition, when K⁺-channel activity in cimetidine-inhibited tissues was blocked with either 1 or 5 mM Ba²⁺, R increased to 126.0 ± 5.0% and 140.0 ± 7.0%, respectively, of the initial R (data not shown).

DISCUSSION

The present study shows that basolateral exposure of gastric oxyntic cells to as little as 92.5 μM NH₃ present in 10 mM NH₄Cl at pH 7.2, completely inhibits acid secretion within 30 min. Similar concentrations of NH₃ could permeate the gastric mucosa during acute infection with HP through defects in the epithelium or...
circulate in the blood of patients with chronic renal failure or hepatic disease. This inhibition of acid secretion with NH₄Cl is more than three times faster than with other acid inhibitors such as cimetidine (H₂ receptor agonist) or omeprazole (proton pump inhibitor). Because NH₄Cl inhibits acid secretion after stimulation with histamine plus carbachol or forskolin, it is likely that NH₄Cl acts at a site distal to H₂ receptor binding and to the activation of adenylate cyclase. Inhibition of acid secretion by NH₄Cl was accompanied by an increase in PD and R nearly identical in magnitude to the increase in PD and R that occurred during inhibition of acid secretion by either cimetidine or omeprazole.

Our results using 10 mM NH₄Cl to inhibit acid secretion are consistent with those of Yanaka et al. (40) who showed that 30 mM nutrient NH₄Cl (270 μM NH₃) completely inhibits stimulated acid secretion in the frog. Whereas the NH₄Cl-induced inhibition of acid secretion in the present study was completely reversed after withdrawal of NH₄Cl, Yanaka et al. (40) showed only minimal recovery after withdrawal of NH₄Cl. These results suggest that the ability of acid secretion to recover fully after an acute challenge with NH₄Cl is concentration dependent. The finding that the morphology of oxyntic cells remained stimulated despite the absence of acid secretion accounts for the ability of these cells to regain nearly normal rates of acid secretion rapidly after the withdrawal of NH₄Cl from the nutrient solution; reassembly of the apical surface takes nearly 2 h from the inhibited configuration in frog gastric mucosa (10).

The way in which NH₃ or NH₄⁺ enters the basolateral membrane of gastric oxyntic cells is not known. However, the basolateral membrane of gastric oxyntic cells possess multiple permeability pathways for Na⁺ and K⁺ (Na⁺/H⁺ exchange, Na⁺-HCO₃ cotransport, Na⁺-K⁺-2Cl⁻ cotransport, Na⁺-K⁺-ATPase) that could transport NH₄⁺ into the cell. Our results demonstrate, however, that basolateral ion transporters do not facilitate the rapid movement of NH₄⁺ into gastric oxyntic cells because blockade of basolateral ion transport activity did not mitigate the effect of NH₄Cl on acid secretion. It is most likely that NH₃ enters cells at the basolateral membrane by passive diffusion as was postulated by Yanaka et al. (40) and then is rapidly converted to NH₄⁺ in the cytoplasm at neutral pH. The results presented here differ from those obtained in the kidney where NH₃ is moved rapidly into cells by renal Na⁺-K⁺-ATPase or by Na⁺-K⁺-2Cl⁻ cotransporter activity depending on cell type (15).

Several lines of evidence from the present study suggest that NH₄Cl does not block acid secretion by blockade of basolateral ion transport activity. We show that inhibition of Na⁺/H⁺ exchange with amiloride, Na⁺-K⁺-ATPase with ouabain, or Na⁺-K⁺-2Cl⁻ cotransport with bumetanide for 30 min has little effect on stimulated acid secretion in the frog gastric mucosa. These results are consistent with other reports (11, 28) in which blockade of transport activity had little effect on stimulated acid secretion in the frog. Thus even if NH₄Cl inhibited the activity of one of these three exchangers, there would be no demonstrable effect on acid secretion. In contrast, our results show that acid secretion in the frog gastric mucosa was partially, but not completely, inhibited by blockade of Na⁺-HCO₃ cotransport and/or Cl⁻/HCO₃⁻ exchange with DIDS. Similar results were obtained with DIDS in the rat, mouse, and rabbit gastric mucosa (11, 12, 22). The finding that acid secretion can be inhibited by blockade of Na⁺-HCO₃ cotransport and/or Cl⁻/HCO₃⁻ exchange suggests that NH₄Cl could reduce acid secretion, in part, by blockade of one or both of these basolateral transporters. Against this possibility is the finding that the addition of NH₄Cl to tissues incubated with DIDS immediately reduced acid secretion to 0, suggesting that NH₄Cl and DIDS act at different sites. Thus it is unlikely that NH₄Cl inhibits acid secretion by blocking Na⁺-HCO₃ cotransport and/or Cl⁻/HCO₃⁻ exchange in gastric oxyntic cells.

Imidazole can be used to mimic the effects of NH₃ as a weak base. Like NH₃, an equimolar concentration of imidazole should result in a similar amount of cytosolic alkalization and neutralization of acidic intracellular compartments due to its ability to easily permeate cell membranes (19). However, when acid secretion was measured for 3 h in the presence of 200 μM nutrient imidazole at pH 7.1 (100 μM imidazole vs. 92.3 μM NH₃), the rate of acid secretion was not significantly different from that of control tissues. Furthermore, acid secretion in 3 h was inhibited only slightly from 6.6 to 4.03 μeq · h⁻¹ · cm⁻² in the presence of 10 mM nutrient imidazole at pH 7.1 (5 mM imidazole). The finding that 10 mM nutrient NH₄Cl inhibited acid secretion immediately in the presence of either 200 μM or 5 mM imidazole at pH 7.1 suggests that the action of NH₃ and imidazole are different. A similar conclusion was drawn from studies using NH₄Cl, imidazole, and other weak bases in intestinal T84 cells (27).

Methylamine, a C₁ primary alkyamine that is larger and more lipophilic than NH₃ (C₀ primary alkyamine), can be used to test the effects of related primary alkyamines on acid secretion. Our results clearly demonstrate that methylamine rapidly inhibits acid secretion in the frog gastric mucosa. Because of the larger size and lipophilicity, a larger concentration of methylamine was required to inhibit acid secretion as effectively as with NH₄Cl. In addition, acid secretion did not recover fully after inhibition with methylamine because methylamine will not exit cells as rapidly as does NH₄Cl. Our results are consistent with those by Hrnjez et al. (14), who showed that related primary alkyamines including C₀ (ammonium) to C₈ (octylamine) inhibited stimulated Cl⁻ secretion in intestinal T84 cells. Because alkyamines were shown to inhibit Cl⁻ secretion by blocking resting basolateral K⁺-channel activity (14), we considered the possibility that NH₄Cl and methylamine inhibit K⁺-channel activity in gastric oxyntic cells.

Our data show, however, that exposure of the gastric mucosa to NH₄Cl does not block acid secretion by blockade of basolateral K⁺-channel activity. From the
data presented here and by others (18, 25, 33–35), it is clear that blockade of basolateral K+-channel activity does not significantly influence the rate of stimulated acid secretion in the gastric mucosa. The present study shows that 1 h or more of incubation with Ba2+ (5 mM) is required to significantly reduce the rate of stimulated acid secretion in the frog gastric mucosa, and by 3 h acid secretion was reduced only 44% compared with control tissues. In addition, inhibition of acid secretion does not occur in 3 h when the Ba2+ concentration is reduced to 0.5 or 1 mM. These results are consistent with those previously reported by Schwartz et al. (35) and McLennan et al. (18), who incubated the gastric mucosa for 10–20 min in Ba2+ and saw little effect on stimulated acid secretion at concentrations <5 mM. Incubation of stimulated tissues with Ba2+ also caused a significant increase in R with little to no influence on PD. These results are consistent with those previously reported in both frog and piglet mucosa (18, 25, 33–35). Inhibition of K+-channel activity with tobutamidine, clotrimazole, or TEA had no effect on acid secretion, R, or PD, suggesting that these K+-channel blockers are not effective in the frog gastric mucosa. Similar conclusions were reported recently (5) for tolbutamide in rabbit gastric glands.

The finding that an increase in R of the gastric mucosa incubated with NH4Cl occurs only in stimulated tissues suggests that NH4Cl acts to specifically inhibit some aspect of stimulated acid secretion. In addition to numerous basolateral K+ channels that recycle K+ imported by Na+-K+-ATPase, it is thought that oxyntic and parietal cells possess an apical K+ channel(s) that is (are) active only during acid secretion. The apical K+ channel facilitates the movement of cytoplasmic K+ into the gastric lumen; K+ is then recycled back into the cell by H+-K+-ATPase activity during acid secretion (7). In a preliminary report by Horio et al. (13), it was determined that the apical membrane of stimulated parietal cells has an inwardly rectifying K+ channel (Kir4.1). We propose that it is this K+ channel that is blocked by NH4Cl and methylamine. If so, the apical K+ channel represents an important regulatory site for activity of gastric H+-K+-ATPase. Interestingly, it has been shown (6) that NH4+ is a potent inhibitor of Ca2+-dependent inwardly rectifying K+-channel activity in HeLa cells. Studies that address the relationship of NH4+ and apical K+-channel activity in gastric oxyntic and parietal cells are important and merit further investigation.

If an apical K+ channel is blocked by NH4Cl, it is important to explain 1) why only NH3 and not other K+-channel blockers inhibit acid secretion and 2) why NH3 and Ba2+ do not effectively block acid secretion when administered from the luminal solution. We believe it is likely that NH4Cl blocks acid secretion from the cytosolic face of the K+ channel as was described for inhibition (by NH4Cl) of the inwardly rectifying K+ channel in HeLa cells (6). Thus NH4Cl can get into cells and to the cytosolic aspect of the apical membrane by virtue of its lipophilicity but Ba2+ (and other K+-channel blockers) cannot. This hypothesis is consistent with studies (29, 39) that showed isolated (apical) membrane vesicles have a Ba2+-inhibitable K+-conductance whereas luminal Ba2+ (up to 20 mM) had no effect on acid secretion in the intact frog gastric mucosa (35). These studies suggest that Ba2+ can effectively block an apical K+-conductance when the apical membrane is isolated from the intact cell. It is not clear to us why NH4Cl does not inhibit acid secretion from the apical surface. It may be due to the lack of permeability of the apical membrane to NH4Cl (2) or may be because the secretory “flush” during acid secretion makes it difficult for substances to interact directly with the apical membrane. Further work is needed to understand, in detail, the effects of NH4Cl on acid secretion in intact tissues.

Our hypothesis concerning the action of NH3 on gastric oxyntic cells is not consistent with that set forth by Fryklund et al. (8). In that study (8), aminopyrine uptake, oxygen consumption, and glucose oxidation were shown to increase in isolated and stimulated glands (rabbit) and decrease in inhibited glands. Because glucose oxidation also increased in the presence of NH4+, it was suggested that NH3 stimulates H+-K+-ATPase activity. It was proposed that NH3 enters the canalicular membrane and is protonated by secreted H+ to form NH4+, and then NH4+ acts as a surrogate for K+ on the active H+-K+-ATPase. We cannot rule out the possibility that titration of secreted H+ by NH3 and recycling of NH4+ is the mechanism by which NH4Cl inhibits acid secretion. However, because the flux of a related primary alkylamine (methylamine) is much slower than the measured rate of acid secretion in stimulated tissues, it seems unlikely that titration and recycling can account for a rapid and complete inhibition of acid secretion in gastric tissues. Because of the effect of NH4Cl on R in inhibited and stimulated tissues and the inhibition of acid secretion by related alkylamines that do not interact with H+-K+-ATPase, we believe that the K+ channel hypothesis warrants further investigation.

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REFERENCES
G410 INHIBITION OF ACID SECRETION BY NH₄Cl


9. G160 INHIBITION OF ACID SECRETION BY NH₄Cl


