Ammonia blockade of intestinal epithelial K⁺ conductance

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Hrnjez, Bruce J., Jae Kyung C. Song, Madhu Prasad, Julio M. Mayol, and Jeffrey B. Matthews. Ammonia blockade of intestinal epithelial K⁺ conductance. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G521–G532, 1999.—Ammonia profoundly inhibits cAMP-dependent Cl⁻ secretion in model T84 human intestinal crypt epithelia. Because colonic luminal concentrations of ammonia are high (10–70 mM), ammonia may be a novel regulator of secretory diarrheal responsiveness. We defined the target of ammonia action by structure-function analysis with a series of primary amines (ammonia, methylamine, ethylamine, propylamine, butylamine, pentylamine, hexylamine, heptylamine, and octylamine) that vary principally in size and lipid solubilities. The amine concentrations required for 50% inhibition of outward K⁺ current (Iₒ) in apically permeabilized monolayers vs. the Iₒ of the respective amine partition coefficients give two plots that are strikingly similar in character. Half-maximal inhibition of short-circuit current (Iₛ), by ammonia was seen at 6 mM and for Iₒ, at 4 mM; half-maximal inhibition for octylamine was 0.24 mM and 0.19 mM for Iₒ and Iₛ, respectively. The preferentially water-soluble hydrophilic amines (ammonia, methylamine, ethylamine) increase in blocking ability with decreasing size and lipophilicity. Conversely, the preferentially lipid-soluble hydrophobic (propylamine, butylamine, pentylamine, hexylamine, heptylamine, octylamine) amines increase in blocking ability with increasing size and lipophilicity. Ammonia does not affect isolated apical Cl⁻ conductance; amine-induced changes in cytosolic and endosomal pH do not correlate with secretory inhibition. We propose that ammonia in its protonated ammonium form (NH₄⁺) inhibits cAMP-dependent Cl⁻ secretion in T84 monolayers by blocking basolateral K⁺ channels.

Ammonia; chloride; diarrhea; T84 cells; pH

Ammonia and related amines are normal products of cellular transamination and deamination reactions. The renal medulla contains a high level of ammonia, which is ultimately eliminated in the urine in the form of urea, the chief final product of nitrogen metabolism in mammals. The concentration of ammonia in most other healthy organs is quite low; levels exceeding 1 mM are usually toxic to mammalian cells. It is well known, however, that the lumen of the lower gastrointestinal tract is the setting for bacterial action on ingested protein and that the colon consequently experiences concentrations of the protein degradation product ammonia that may reach 100 mM. Little is known about the impact of such levels of ammonia on normal intestinal epithelial cell function (1, 3, 7, 13, 22, 38). We recently reported that ammonia markedly inhibits cyclic nucleotide-dependent Cl⁻ secretion in the T84 human intestinal cell model of secondary active epithelial Cl⁻ transport (22, 38) and proposed that luminal ammonia may be an endogenous negative regulator of colonic secretion, serving to dampen epithelial responsiveness to potentially diarrheagenic stimuli in the bacteria-rich lumen. We were unable at that time to identify the specific target of ammonia action but concluded that it is distal to cAMP generation. Indirect evidence indicated that ammonia action is independent of cytosolic alkalinization.

Here, we report that this inhibition results from NH₄⁺ block of the basolateral membrane K⁺ conductance. This observation runs counter to the conventional attribution of ammonia's biological action to the ability to alter cytosolic or endosomal pH (1, 3, 7, 13). It also runs counter to the well-known ability of many K⁺ channels and transport pathways to support NH₄⁺ movement (25). Recognition that NH₄⁺ can block K⁺ channels may well be important to understanding regulated ion transport in organ systems exposed to high levels of ammonia under normal circumstances, in disease states such as cirrhosis (32) and chronic renal insufficiency (11, 35, 49) and during infections with urealytic organisms such as Helicobacter pylori (6).

In accord with observations (12) on the action of ammonia and several lysosomotropic amine weak bases on Dictyostelium discoideum, preliminary experiments in our laboratory showed that the same amines also suppress Cl⁻ secretion in T84 cells and that the ability to do so may increase with increasing amine lipophilicity. Such compounds have been observed in many instances to perturb plasma membrane recycling, an effect that is widely assumed to be due to their ability to accumulate within and raise the pH of acidic endosomal compartments (13). Furthermore, recent evidence (4, 5, 39) suggests that cAMP-dependent regulation of transepithelial Cl⁻ secretion may involve rapid insertion or retrieval of membrane vesicles containing ion channels such as cystic fibrosis transmembrane conductance regulator. This finding initially led us to speculate that the effect of ammonia on T84 cell secretion could be due to perturbation of endosomal pH and, consequently, the regulated insertion or retrieval of key transport proteins involved in transepithelial Cl⁻ transport. However, the variations in structural, functional, and chemical properties of the amines we chose to

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examine it made difficult to draw conclusions about a common mode of action. Moreover, we recently noted (33) some similarities in ammonia’s effect on epithelial transport to that of Ba2+, a well-known K+ channel blocker, and could not easily reconcile this finding with the membrane-recycling hypothesis.

To define more clearly the target of ammonia action, we examined the effect of structural variation about ammonia’s nitrogen center with a series of more closely related amines that have widely varying lipid solubilities (Table 1). We assumed the importance of nitrogen functionality to inhibitory ability and chose amines whose structures, functionalities, and chemical properties vary in a well-defined manner. They comprise the membranelike octanol.

Specifically, we chose these amines to evaluate the relative importance of size, basicity, and lipophilicity to inhibitory ability and then measured their effects on Cl− secretion, agonist-regulated apical membrane Cl− and basolateral membrane K+ conductance, cytosolic pH, endosomal pH, and fluid-phase endocytosis in the T84 cell model.

**EXPERIMENTAL PROCEDURES**

Cell culture and buffers. T84 cells were obtained from the American Type Culture Collection and K. Barrett (Univ. of California, San Diego) and grown to confluence at pH 7.40 in 150-ml flasks with DMEM-Ham’s F-12 nutrient (1:1) and 6% (vol/vol) fetal bovine serum, supplemented with HEPES (1.50 × 10−2 M), NaHCO3 (1.43 × 10−2 M), penicillin G sodium salt (1.70 × 10−4 M), amphotericin B (2.70 × 10−7 M), and streptomycin sulfate (6.86 × 10−3 M). The monolayers were maintained in culture with weekly passage by trypsinization in Ca2+- and Mg2+-free phosphate-buffered solution at a surface ratio of 1:2.

Experiments were carried out in HEPES-phosphate-buffered Ringer solution (HPBR) containing NaCl (1.35 × 10−1 M), KCl (5.0 × 10−3 M), NaH2PO4 (3.33 × 10−3 M), Na2HPO4 (8.30 × 10−4 M), CaCl2 (1.0 × 10−3 M), MgCl2 (1.0 × 10−3 M), glucose (1.0 × 10−2 M), and HEPES (5.0 × 10−3 M). Solutions containing amines (Aldrich Chemical, Milwaukee, WI) were prepared by addition of the hydrochloride salt or the free amine to HPBR without adjustment for variation in osmolarity or Cl− concentration. Control experiments with N-methyl-D-glucamine (NMG) demonstrated that hyperosmolar apical or basolateral solutions slightly suppressed secretion only at high concentrations (>40 mM). All solutions were used at room temperature and adjusted to a pH of 7.40 (with HCl or NaOH) unless otherwise specified.

Transepithelial Cl− transport. Dose-response curves for suppression of Cl− secretion with the amines in Table 1 were obtained with T84 cells seeded and grown to confluence on collagen-coated permeable supports with an area of 0.33 cm2 (24-well inserts, Costar, Cambridge, MA). Monolayers were fed every 2–3 days and the night before the experiment and were used between 7 and 14 days after they were plated. Transepithelial potential difference and short-circuit current (Isc) were measured with a dual voltage-current clamp (model 616C-2, Univ. of Iowa, Iowa City, IA) with apical and basolateral Ag-AgCl and calomel electrodes connected via “chopstick” KCl-agar bridges (38). This setup facilitated the performance of large numbers of transport studies that would have been substantially more cumbersome in traditional Ussing chamber models. Forskolin-stimulated Isc at room temperature is approximately equivalent to that obtained at 37°C in this model, and the effects of ammonia and amines were similar (not shown). Typically, electrical measurements were made at 0 min on monolayers in HPBR after they were washed free of medium, after a 30-min equilibration in HPBR at room temperature, after a 30-min equilibration with or without amine, and 15 and 45 min after basolateral stimulation with forskolin (1.0 × 10−5 M; Calbiochem, La Jolla, CA). Each reported value for transepithelial resistance and Isc is the average of n = 4 measurements.

Permeabilized system. The outward K+ current (I K) was measured, and dose responses were conducted for basolaterally applied amines 1–9 to monolayers apically permeabilized by nystatin (23, 28, 52). T84 cells grown to confluent monolayers (resistance of >900 Ω·cm2) on collagen-coated Snapwell inserts (0.4-mm pore size, 12 mm diameter, Costar) were washed once with a low K+–low Na+, glucose solution (1.0 × 10−2 M sodium gluconate, 1.35 × 10−1 M NMG-glucronate, 5.0 × 10−2 M potassium gluconate, 1.0 × 10−3 M MgSO4, 1.0 × 10−3 M calcium gluconate, 1.0 × 10−2 M glucose, and 1.0 × 10−2 M HEPES at pH 7.40). The monolayers were mounted in an Ussing-type diffusion chamber system (model DCCSYS, Precision Instrument Design, Tahoe City, CA) interfaced with computer-based data acquisition software (MacoLab System Chart v3.5.4) and a multichannel voltage-current clamp (model VCC MC6 revision A, Precision Instrument Design). Apical and basolateral chambers contained the wash solution described above during 15-min monolayer equilibration at 37°C. Current as a function of time was measured with the voltage clamped at 0 mV. Voltage pulses of 1-s duration and 10-mV magnitude were passed across the monolayer at 10-s intervals to allow determination of transepithelial resistance. The apical chamber solution was replaced with a high K+–low Na+, glucose solution (1.0 × 10−2 M sodium gluconate, 1.4 × 10−3 M potassium gluconate, 1.0 × 10−2 M MgSO4, 1.0 × 10−3 M calcium gluconate, 1.0 × 10−2 M glucose, and 1.0 × 10−2 M HEPES at pH 7.40) to apply an apical-to-basolateral K+ gradient across the monolayer. Nystatin (500 U/ml; Sigma, St. Louis, MO) was added to the apical solution to permeabilize the apical membrane to small monovalent ions. Cells were treated with ouabain (1.0 × 10−4 M, Sigma) to inhibit the basolateral 3Na+-2K+-ATPase and prevent its contribu-

**Table 1. Amine series**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amine</th>
<th>Chemical Chemical</th>
<th>pK&lt;sub&gt;α&lt;/sub&gt;</th>
<th>Log P</th>
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<tr>
<td>1</td>
<td>Ammonia</td>
<td>NH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>9.24</td>
<td>−2.04</td>
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<td>2</td>
<td>Methylamine</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10.66</td>
<td>−0.57</td>
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<tr>
<td>3</td>
<td>Ethylamine</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10.81</td>
<td>−1.97</td>
</tr>
<tr>
<td>4</td>
<td>n-Propylamine</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10.71</td>
<td>−0.37</td>
</tr>
<tr>
<td>5</td>
<td>n-Butylamine</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10.77</td>
<td>−0.88</td>
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<tr>
<td>6</td>
<td>n-Pentylamine</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;4&lt;/sub&gt;NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10.63</td>
<td>+1.05</td>
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<tr>
<td>7</td>
<td>n-Hexylamine</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;5&lt;/sub&gt;NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10.56</td>
<td>+1.52</td>
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<tr>
<td>8</td>
<td>n-Heptylamine</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;6&lt;/sub&gt;NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10.66</td>
<td>+2.02</td>
</tr>
<tr>
<td>9</td>
<td>n-Octylamine</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;7&lt;/sub&gt;NH&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>10</td>
<td>Diethylamine</td>
<td>(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;NH</td>
<td>10.49</td>
<td>−0.57</td>
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<tr>
<td>11</td>
<td>Triethylamine</td>
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<td>10.11</td>
<td>−1.44</td>
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<tr>
<td>12</td>
<td>Trimethylamine</td>
<td>(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;N</td>
<td>9.81</td>
<td>−0.27</td>
</tr>
<tr>
<td>13</td>
<td>Tri-n-butylamine</td>
<td>(CH&lt;sub&gt;3&lt;/sub&gt;(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;N</td>
<td>10.70</td>
<td>−1.52</td>
</tr>
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</table>

pK<sub>α</sub> refers to the pKa of the conjugate acid of these basic amines. Log P represents the partition coefficient (P) of a given solute between immiscible octanol and water in intimate contact. That is, P represents the molar ratio of solute dissolved in octanol to that dissolved in water. Increasingly negative log P indicates increasingly preferential solubility in water. Increasingly positive log P indicates increasingly preferential solubility of the solute in the lipophilic membranelike octanol.

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tion to the \( I_K \). The resultant \( I_K \) through basolateral \( K^+ \) channels was thereby isolated and measured. Small aliquots of a concentrated solution of the appropriate amine from Table 1 were added to the basolateral solution, and the effects on \( I_K \) were observed and recorded. In some cases, carbachol (\( 1.0 \times 10^{-5} \) M, Sigma) was added to determine amine effects on Ca\(^{2+}\)-sensitive \( K^+ \) channels when the Ca\(^{2+}\)-independent or “resting” \( I_K \) reached zero after amine treatment. These apical permeabilization experiments were conducted in Cl\(^-\)-free solutions to prevent swelling-induced opening of \( K^+ \) channels in the cell basolateral membrane.

The outward Cl\(^-\) current (\( I_{Cl} \)) through apical Cl\(^-\) channels was measured under similar conditions after the basolateral membrane was permeabilized with nystatin. T84 cells grown to confluency on Snapwell inserts were washed with a low Cl\(^-\)-high K\(^+\) gluconate solution (1.0 \( \times 10^{-2} \) M sodium gluconate, 1.4 \( \times 10^{-1} \) M potassium gluconate, 1.0 \( \times 10^{-3} \) M calcium gluconate, 1.0 \( \times 10^{-3} \) M MgSO\(_4\), 1.0 \( \times 10^{-2} \) M glucose, and 1.0 \( \times 10^{-2} \) M NaCl at pH 7.40). Monolayers were mounted in the same diffusion chamber system as described above for \( I_K \) measurement. The basolateral chamber contents were replaced with a high Cl\(^-\)-high K\(^+\) solution (1.0 \( \times 10^{-2} \) M sodium gluconate, 1.4 \( \times 10^{-1} \) M KCl, 1.0 \( \times 10^{-3} \) M CaCl\(_2\), and 1.0 \( \times 10^{-2} \) M HEPES at pH 7.40) to apply a basolateral-to-apical Cl\(^-\) concentration gradient across the monolayer. Nystatin (500 U/ml) was added to the basolateral solution to permeabilize the basolateral membrane to small monovalent ions. Forskolin (1.0 \( \times 10^{-5} \) M) was added to the apical solution to stimulate the Cl\(^-\) secretion through apical Cl\(^-\) channels, and the isolated cAMP-stimulated \( I_{Cl} \) was measured and recorded. Several experiments with amines 1–9 were performed with the appropriate amine added to the basolateral and/or apical solutions to test for direct effects on apical Cl\(^-\)-conductance.

Cytosolic pH. Polarized T84 cells grown to confluency on Anocell inserts (surface area = 0.33 cm\(^2\); Whatman, Maidstone, UK) were used to fluorometrically (Spectro DM3000, Spex Industries, Edison, NJ) measure cytosolic pH and its response to separate apical and basolateral application of the amines 1–9 in Table 1 (42). Cell monolayers were loaded with the acetoxymethyl ester of 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF AM, 2.0 \( \times 10^{-7} \) M in HPBR with glucose; Molecular Probes, Eugene, OR) for 1 h at room temperature and then mounted in a modified spectroscopic quartz cuvette that afforded separate access to the isolated apical and basolateral aspects of the confluent monolayers. Small aliquots of a concentrated solution of the appropriate amines 1–9 were added to the apical and/or basolateral solution, and the effects on intracellular pH were observed and recorded as a function of time.

The quartz cuvette was modified with a clear, colorless, and flat piece of Lucite (thickness = 1.0 mm) that was cut (width = 10.0 mm, height = 65.0 mm) so as to fit snugly a quartz fluorescence cuvette of standard internal dimensions (width = length = 10.0 mm, height = 43.0 mm). A single hole (diameter = 3.0 mm, centered at height = 13.0 mm), intended to coincide with the incident excitation light beam, was drilled in the cut Lucite. When inserted into the cuvette, the plane of the flat Lucite is perpendicular to the sides, parallel to the front and rear faces, and bisects the distance between the front and rear faces, affording a cuvette with equal volume front (apical) and rear (basolateral) compartments. The seal between the edges of the Lucite insert and the inner walls of the cuvette was accomplished with fingertip rope caulking (Frost King, Paterson, NJ). Sample, in the form of a T84 monolayer loaded with dye on an irregular flat fragment of an optically clear and rigid Anocell-permeable support, was placed in the cuvette, cell side up, on the Lucite insert so as to completely the 3-mm-diameter drilled hole. The seal between the bottom surface of the permeable support and the Lucite surface surrounding the hole was made with a thin film of high-vacuum silicone grease (Dow Corning, Midland, MI). The cuvette was consistently inserted in the fluorometer sample compartment so that the excitation beam initially struck the apical rather than the basolateral aspect of the monolayer. The monolayer surface was perpendicular to the excitation beam, and fluorescence was collected at an angle 45° to the incident beam (front-face mode). The sample was excited at 505 and 439 nm. Separate emission intensities were collected at 535 nm as a function of time (data point interval = 4 s) and ratioed (ratio of 505-nm to 439-nm excitation) after data collection. Excitation and emission slits were set at 0.5 mm, corresponding to a bandpass of 1.8 nm.

The emission ratio at 535 nm was correlated with pH via intracellular calibration. The sodium salt of the polyether nigericin (0.1 ml of a stock solution containing 10 mg in 1.0 ml dimethylformamide and 0.3 ml ethanol) in high-K\(^+\) buffer (40 ml, the same as the aforementioned HPBR except the concentrations of Na\(^+\) and K\(^+\) are reversed) at 2.6 \( \times 10^{-5} \) M was used to bilaterally permeabilize the cell membrane to the ions H\(_2\)O\(^+\) and K\(^+\). Monolayers loaded with dye and mounted normally were sequentially and bilaterally exposed to the high-K\(^+\) nigericin buffer at five different pH values between 6.60 and 8.00, and the respective emission ratios were measured after an ~15-min equilibration of the intracellular and extracellular K\(^+\) and H\(_2\)O\(^+\) concentrations. The results from several different monolayers were averaged, and a plot of pH vs. emission ratio \((y = 3.06 + 0.58x; r = 0.98)\) afforded a calibration curve.

Endosomal pH. The average pH of a broad population of endosomal compartments was also measured fluorometrically (Spectro DM3000). T84 cells grown to confluency in a 163-cm\(^2\) flask containing the cell culture medium (30 ml) described above were loaded with fluorescein isothiocyanate-dextran (FITC-dextran, molecular weight of 12,000, with 0.73 mol fluorescein/mol dextran, 15.0 mg/ml; Sigma) at 37°C for 24 h (37). The cells were then washed (3 \( \times 30 \) ml at pH 7.40 and 37°C) with PBS (8.0 \( \times 10^{-3} \) M Na\(_2\)HPO\(_4\), 1.5 \( \times 10^{-3} \) M CaCl\(_2\), and 0.9 M KCl, pH 7.40, quenched with medium (30 ml), and centrifuged (1,200 rpm, 5 min at room temperature). The resultant cells were washed with HPBR (3 \( \times 30 \) ml) and then finally suspended at pH 7.40 in HPBR (10 ml). Aliquots (1 ml) of this dye-loaded cell suspension were diluted to 3 ml with HPBR in a standard quartz fluorescence cuvette and magnetically stirred at room temperature during data collection. Small aliquots of a concentrated solution of the appropriate amines 1–9 were added to the stirred cell suspension, and the effects on endosomal pH were observed and recorded.

Fluorescence was collected at an angle of 45° to the incident beam (front-face mode). The sample was excited at 495 and 450 nm. Separate emission intensities were collected at 519 nm as a function of time (data point interval = 4 s) and ratioed (ratio of 495-nm to 450-nm excitation) after data collection. Excitation and emission slits were set at 0.5 mm, corresponding to a bandpass of 1.8 nm.

The emission ratio at 519 nm was correlated with pH by in vitro extracellular calibration. FITC-dextran (3.0 mg, \( \sim 10^{-7} \) mol fluorescein) was dissolved in HPBR (1.0 liters). Fifteen aliquots of this solution were adjusted to different pH values between 5.18 and 6.87, and the respective emission ratios were measured. A plot of pH vs. emission ratio afforded a calibration curve that was linear from pH 5.18 to 6.37 (\( y = -5.14 + 1.44x; r = 0.99 \)) but nonlinear in the region from 6.47
to 6.87. This nonlinear region was approximated effectively as a line ($y = 0.037 + 0.52x; r = 0.96$).

Fluid-phase endocytosis. The apical or basolateral medium solution of T84 cells grown to confluence on collagen-coated permeable supports (area = 4.70 cm$^2$, 3.0-µm pore size) was replaced with medium containing FITC-dextran (molecular weight of 12,000, with 0.73 ml fluorescein/mg dextran, 15.0 mg/ml, Sigma) and incubated for 6 min at 37°C. Immediately after incubation, each monolayer was immersed in a large volume (250 ml) of HPBR at 4°C and then washed twice more by immersion for 30 s in fresh HPBR at 4°C. Residual liquid was aspirated from the edges of the filter membrane, which was then excised from its plastic support and inserted into an Eppendorf tube (1.5 ml capacity) containing distilled water (0.43 ml). The contents of the tube were sonicated (model 550 sonic dismembrator, setting 3, Fisher Scientific). The porous membrane was removed, and the remaining suspension was freed of solid cellular debris by centrifugation (2 × 5 min at 14,000 g). The resultant clear solution was analyzed fluorometrically (excitation wavelength = 495 nm, emission wavelength = 565 nm) for its relative FITC content. Complete suppression of FITC-dextran uptake at 4°C supports this technique as a general measure of pinocytotic behavior. Rubidium ion efflux. T84 cells grown to confluence on collagen-coated permeable supports (area = 0.33 cm$^2$) were washed free of medium and placed in HPBR. Cells were loaded basolaterally with $^{86}$Rb$^+$ (1.5 mCi/ml in HPBR) at 37°C for 2 h and then washed free of radioisotope-containing solution with HPBR. Baseline efflux was measured for 5 min, and then monolayers were simultaneously and basolaterally exposed to forskolin (10$^{-5}$ M) alone or forskolin plus ammonia (amine 1; 4.0 × 10$^{-3}$ M), methylamine (amine 2; 1.0 × 10$^{-2}$ M), or octylamine (amine 9; 7.0 × 10$^{-4}$ M). Data points were collected as aliquots from the basolateral bath solution every 2 min and counted in Atomlight (Packard, Meriden, CT) scintillation fluid with a liquid scintillation analyzer (Packard model 1600 TR).

Statistical analyses. Data are reported as means ± SE. Analysis included the Student’s t-test for paired or unpaired variables and two-way ANOVA when appropriate, with $P < 0.05$ considered significant.

RESULTS

Characteristics of amines. The amines listed in Table 1 share the presence of a single formally sp$^3$ hybridized nitrogen. Entries 2 through 9 are straight-chain primary alkyl amines that vary according to the number of methylene units (—CH$_2$—) in the single alkyl group attached to the primary amine (—NH$_3$—) functionality. Together, they represent a tool designed to elucidate the means by which ammonia suppresses Cl$^-$ secretion in T84 monolayers. They vary most obviously in size, that is, length, of the lipophilic alkyl tail bonded to the nitrogen that they hold in common with ammonia (amine 1). As evidenced by the relatively invariant $pK_a$ values of their conjugate acids ($pK_{aH^+}$) in water (50), successive addition of methylene groups for 2 through 9 has little effect on their relative basicities. This is because the electron-donating character of higher-order alkyl chains is about the same as that of methyl (amine 2). Electronically, series 2–9 (average $pK_{aH^+} = 10.68$) is uniform in its difference from the parent unsubstituted ammonia ($pK_{aH^+} = 9.24$) with respect to the nitrogen center. Likewise, the aqueous nucophile-reactivity of the unprotonated amines is approximately invariant in amines 2–9. Nucleophilicity often parallels basicity, and the steric environment in the immediate vicinity of the active nitrogen center changes little from methylamine (amine 2) through n-octylamine (amine 9). This uniformity in electronic and steric character at nitrogen in primary amines 2–9 further suggests that the intermolecular interactions between solvent water molecules and the —NH$_3$— functionality are similarly uniform. Amines 2–9 therefore present a uniform nitrogen “head” but nonuniform alkyl “tails” to the target inhibitory site (8, 30).

Alkyl amines suppress Cl$^-$ secretion. We measured the $I_{sc}$ responses to CAMP in the presence or absence of amines 1–9 over a range of concentrations. Representative, Fig. 1 depicts a small portion of the data for the two apparent extremes in the amine series. Basolaterally applied ammonia (amine 1) in Fig. 1A gives the previously reported diminution in forskolin-stimulated $I_{sc}$ (a measure of Cl$^-$ secretion in epithelial monolayers) relative to control monolayers (22). Basolaterally applied octylamine (amine 9) in Fig. 1B similarly inhibits $I_{sc}$ but requires considerably less of this lipophilic amine to almost completely suppress secretion. Pretreatment with amine has no effect on baseline $I_{sc}$ before forskolin stimulation. Figure 2 illustrates that the concomitant sharp drop in transepithelial resistance with administration of forskolin is markedly blunted by amines 1 and 9, and amine pretreatment also has no effect on baseline transepithelial resistance. Ammonia and alkyl amines suppress basolateral K$^+$ conductance. Transepithelial Cl$^-$ transport by T84 and many other secretory epithelial cells requires the integrated activity of four membrane-bound systems: apical Cl$^-$ channels and a basolateral machinery consist-

![Image](http://ajpgi.physiology.org/download/figshare/10.2203.31.1.017_251173/fig1.jpg)
ing of the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter, the 3Na\(^+\)-2K\(^+\)-ATPase, and K\(^+\) channels. We previously noted a parallel between the effect of NH\(_4\)\(^+\) and the effect of Ba\(^{2+}\), a well-known K\(^+\) channel blocker, on cAMP- and Ca\(^{2+}\)-regulated transepithelial Cl\(^-\) secretion and speculated that the basolateral K\(^+\) channel could be a target of action (33). The monovalent cations NH\(_4\)\(^+\) and K\(^+\) are of similar size; their Pauling ionic radii are 1.48 and 1.33 Å, respectively (9). Because the positively charged NH\(_4\)\(^+\) is likely to compete with other positively charged species for electron-rich sites, we reasoned that the cationic NH\(_4\)\(^+\) may interfere with the secretory machinery at a site with high affinity for cations of similar size and electronegativity (24).

Accordingly, we used apically permeabilized, ouabain-inhibited T84 monolayers to isolate and measure the basolateral \(I_K\) and its dose-response to amines 1–9. Representatively, Fig. 3 depicts block of resting \(I_K\) by basolaterally applied ammonia and octylamine, respectively. In our hands, this resting \(I_K\), and its block by amines, is insensitive to the cAMP agonist forskolin, and results were similar in the presence or absence of a prior cAMP stimulus. For clarity, we show only portions of the curves subsequent to application of the apical-to-basolateral K\(^+\) gradient, treatment with ouabain, and permeabilization of the apical membrane with nystatin. The \(I_K\) typically remained stable after nystatin permeabilization without substantial rundown over the course of a 20- to 30-min experiment. The transepithelial voltage pulses from 0 to 10 mV for calculation of transepithelial resistance as a function of time also are omitted for clarity. These amines and the remaining entries (2–8) block outward \(I_K\) on the same order of magnitude as they block \(I_{SC}\), suggesting that they may share a common target. Ammonia and selected alkyl amines also inhibited basolateral \(^{86}\)Rb\(^+\) efflux in intact monolayers. As shown in Fig. 4, efflux was suppressed by >50% relative to control under forskolin-stimulated conditions in the presence of basolateral ammonia (amine 1; 8 mM), methylamine (amine 2; 10 mM), and octylamine (amine 9; 0.8 mM).

Ammonia does not alter cAMP-regulated apical Cl\(^-\) conductance. Basolateral permeabilization of monolayers with nystatin allows us to isolate and study apical Cl\(^-\) conductance in the same way that apical permeabilization with nystatin allows isolation and study of basolateral K\(^+\) conductance. We determined that basolaterally applied ammonia does not affect forskolin-stimulated (cAMP-mediated) apical Cl\(^-\) con-
ductance in this permeabilized system. For example, a representative monolayer (of n = 4) gave a baseline $I_{Cl}$ of 9 µA with a basolateral-to-apical Cl⁻ concentration gradient of 140:5 mM. Forskolin stimulation sharply increased $I_{Cl}$ to a stable 96.5 µA. Basolateral application of 30 mM NH₄Cl left $I_{Cl}$ virtually unchanged at 97.5 µA. Ammonia interference with the cAMP-regulated component of the apical Cl⁻ conductance is therefore unlikely. We did not examine whether amines also inhibited the baseline $I_{Cl}$ of nystatin-permeabilized monolayers and thus cannot determine whether cell swelling-induced Cl⁻ currents are affected.

Lipophilicity is the key variable relating inhibition of Cl⁻ secretion and basolateral K⁺ conductance. The similarity of $I_{sc}$ and $I_{K}$ responses to lipophilic amines 4–9 is underscored by the summarized linear regions of the dose-response curves for $I_{sc}$ and $I_{K}$ from the least lipophilic, propylamine (amine 4), to the most lipophilic, octylamine (amine 9). The slopes within the $I_{sc}$ subset (Fig. 5A) are similar (average slope $= -0.28 \pm 0.02$) as are those within the $I_{K}$ subset (average slope $= -0.42 \pm 0.03$). Control of amine probe variables allows us to focus on the change in lipophilicity within series 1–9. This variable approximates solubility within the lipid bilayer of plasma and vesicular membrane environments and is given by the partition coefficient (P) between n-octanol and water (14, 27), conveniently represented as its logarithm (log P). A plot of the amine concentrations at 50% inhibition of $I_{sc}$ ($\%_{is}I_{sc}$) vs. log P (Fig. 6A) reveals that series 1–9 is organized into two groups, those that are preferentially water soluble (amines 1–3) and those that are preferentially lipid soluble (amines 4–9). The preferentially water-soluble amines increase in ability to block secretion with decreasing size and lipophilicity. Conversely, the preferentially lipid-soluble amines increase in ability to block secretion with increasing size and lipophilicity. This dichotomous behavior reflects a departure from strict adherence to the Meyer-Overton rule (18, 34) for membrane diffusivity. Inhibitory potency does not, therefore, reflect the ability to traverse membranes to reach a cytosolic target or to enter acidic intracellular compartments. The dichotomy is resolved, however, if these two amine subtypes (lipophobic and lipophilic) have essentially the same target but reach it principally by two different routes and interact with the same target in two slightly different modes. This target could be a membrane-bound protein that has a functionally rel-

![Fig. 5. Linear regions of normalized dose responses for lipophilic amines. A: normalized $I_{sc}$ response with respect to natural logarithm of amine 1–9 concentrations. B: normalized $I_{K}$ response with respect to natural logarithm of amine 1–9 concentrations. $I_{0}$ is the $I_{K}$ or $I_{sc}$ in the absence of amine inhibitor.](http://ajpgi.physiology.org/)

![Fig. 6. Intact secretory apparatus response bears striking resemblance to isolated K⁺ current response. A: amine concentrations at 50% inhibition of $I_{sc}$ ($\%_{is}I_{sc}$) as a function of the log of the partition coefficient for basolateral application of amines 1–9 to T84 cell monolayers. B: amine concentrations at 50% inhibition of $I_{K}$ ($\%_{is}I_{K}$) as a function of the log of the partition coefficient for basolateral application of amines 1–9 to monolayers apically permeabilized by nystatin.](http://ajpgi.physiology.org/)
evant lipophobic domain, such as an aqueous pore or transport site. Thus these data are consistent with a membrane-bound ion transport site as such a K⁺ channel as the amine target.

A plot of the amine concentrations at 50% inhibition of $I_\text{K}$ ($50\% I_\text{K}$) vs. log P (Fig. 6B) bears a striking resemblance to the $50\% I_\text{sc}$ functional dependence on amine lipophilicity shown in Fig. 6A. That is, the isolated K⁺ current portion of the secretory apparatus responds with the same character and magnitude as does the intact Cl⁻ secretory apparatus. In particular, ammonia gives a $50\% I_\text{sc}$ of 6.2 mM and a $50\% I_\text{K}$ of 3.9 mM and octylamine gives a $50\% I_\text{sc}$ of 0.24 mM and a $50\% I_\text{K}$ of 0.19 mM.

Dose-response curve fits. Each dose-response curve for the normalized current amplitude ($I_\text{sc}/I_0$ or $I_\text{K}/I_0$) as a function of amine 1–9 concentration was fit to a Langmuir adsorption isotherm (10, 36) of the form

$$\frac{I_x}{I_0} = \frac{1}{1 + \left(\frac{[a]}{K_{1/2}}\right)^n}$$

where [a] is the amine concentration, $K_{1/2}$ is the apparent equilibrium constant for binding of the amine to the discrete target site, n is the Hill coefficient, $I_x$ is $I_\text{K}$ or $I_\text{sc}$, and $I_0$ is the $I_\text{K}$ or $I_\text{sc}$ in the absence of amine inhibitor. When $K_{1/2}$ = [a], the expression $I_x/I_0$ is one-half and [a] corresponds to the dose required for 50% suppression of current. Indeed, data fits to the above equation gave our reported $50\% I_\text{sc}$ and $50\% I_\text{K}$ values. Fits (see Table 2) for $I_\text{sc}$ are consistent with the functional form describing a one-to-one interaction between the amine inhibitor and its target site. Fits for $I_\text{K}$ suggest the possibility of positive cooperativity. However, any apparent cooperativity could potentially be explained by a tendency to overestimate inhibitory potency of amines in the $I_\text{K}$ experiments due to the cumulative dose-response protocol used to obtain these values. This confounding factor was not the case for $I_\text{sc}$ experiments, in which single amine concentrations were used with each monolayer.

Asymmetry and symmetry of apical and basolateral application of amines correlates with amine size and lipophilicity. We previously noted that the inhibitory ability of basolaterally applied ammonia was ~10-fold greater than apically applied ammonia (38). That is, the apical ammonia $50\% I_\text{sc}$ is 50 mM and the basolateral ammonia $50\% I_\text{sc}$ is 5 mM. It was therefore of interest to examine the "sidedness" of the $50\% I_\text{sc}$ values for amines 2–9. In data not shown, apical treatment of T84 monolayers with lipophilic amines 4–9 gives the same $50\% I_\text{sc}$ values as does the basolateral treatment shown in Fig. 6A, but apical treatment with lipophobic amines 1–3 gives $50\% I_\text{sc}$ values fivefold higher than those obtained by basolateral treatment. Thus the sidedness of lipophilic amines 4–9 is symmetrical, but the sidedness of lipophobic amines 1–3 is asymmetrical. Because the compounds were added to only one chamber, this may lead to an underestimation by as much as one-half of the inhibitory constant for the lipophilic subset of amines for both the $I_\text{sc}$ and $I_\text{K}$ experiments.

This correlation of symmetry with lipophilicity is supported by experiments with the highly lipophilic amines 10–13 (Table 1) that lie outside the well-ordered series given by amines 1–9. Amines 10–13 gave about the same $50\% I_\text{sc}$ values for both apical and basolateral application (mean = 27.4, 12.7, 45.3, and 3.3 mM, respectively).

Ammonia and alkyl amine effects on cytosolic pH do not correlate with inhibitory action. When confronted with the observation that ammonia interferes with any aspect of physiological function, the most obvious conclusion is that ammonia is exerting its effect via an ammonia-induced pH change of the cytosolic medium or that of intracellular compartments. Although the foregoing data strongly support the notion that NH₄⁺ blocks K⁺ channels and thereby blocks Cl⁻ secretion, it is also necessary to determine if the ammonia-induced pH change of the cytosolic medium or that of intracellular compartments is related to the block of Cl⁻ secretion. We therefore directly examined the effect of basolateral or apical application of ammonia on cytosolic pH with the knowledge that 10-fold more apical ammonia (50 mM) than basolateral ammonia (5 mM) is needed to suppress Cl⁻ secretion (38). The cytosolic pH responses to apically and basolaterally applied ammonia (30 mM) differ markedly, probably reflecting the permeability differences between the species NH₃ and NH₄⁺ (Fig. 7). Apical application gives an immediate cytosolic pH increase from 7.19 to 7.90 followed by a slow decline to 7.70 after 20 min of continued apical exposure. Basolateral application gives an immediate but tempered increase and relatively rapid recovery to the initial cytosolic pH and below. Apical application likely gives persistent alkalinization because there are limited apical pathways for NH₄⁺ transmembrane movement. The cytosolic pH response to basolateral ammonia (blunted peak alkalinization followed by rapid recovery) resembles that of renal epithelia, in which competitive influx of the protonated NH₄⁺ via the Na⁺-K⁺-2Cl⁻ cotransporter and the 3Na⁺-2K⁺-ATPase has been documented (25).

Inhibition of Cl⁻ secretion does not correlate with these changes in bulk cytosolic pH. Figure 8 depicts the cytosolic pH responses of BCECF-loaded monolayers superimposed on the forskolin-stimulated $I_\text{sc}$ responses

<table>
<thead>
<tr>
<th>Amine</th>
<th>$K_{1/2}$, mM</th>
<th>n</th>
<th>$K_{1/2}$, mM</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.20 ± 0.21</td>
<td>0.74 ± 0.04</td>
<td>3.86 ± 0.12</td>
<td>2.51 ± 0.17</td>
</tr>
<tr>
<td>2</td>
<td>15.95 ± 0.84</td>
<td>0.79 ± 0.10</td>
<td>5.60 ± 0.34</td>
<td>1.61 ± 0.12</td>
</tr>
<tr>
<td>3</td>
<td>29.12 ± 1.96</td>
<td>0.93 ± 0.14</td>
<td>7.24 ± 0.27</td>
<td>2.20 ± 0.15</td>
</tr>
<tr>
<td>4</td>
<td>39.76 ± 2.16</td>
<td>1.49 ± 0.17</td>
<td>15.27 ± 0.87</td>
<td>2.20 ± 0.27</td>
</tr>
<tr>
<td>5</td>
<td>15.47 ± 1.07</td>
<td>1.02 ± 0.15</td>
<td>3.74 ± 0.14</td>
<td>2.35 ± 0.15</td>
</tr>
<tr>
<td>6</td>
<td>9.25 ± 0.34</td>
<td>1.20 ± 0.11</td>
<td>2.69 ± 0.07</td>
<td>1.64 ± 0.06</td>
</tr>
<tr>
<td>7</td>
<td>8.65 ± 0.89</td>
<td>0.79 ± 0.11</td>
<td>1.21 ± 0.03</td>
<td>2.37 ± 0.14</td>
</tr>
<tr>
<td>8</td>
<td>1.06 ± 0.05</td>
<td>1.90 ± 0.17</td>
<td>1.00 ± 0.06</td>
<td>1.68 ± 0.13</td>
</tr>
<tr>
<td>9</td>
<td>0.24 ± 0.01</td>
<td>1.32 ± 0.05</td>
<td>0.19 ± 0.02</td>
<td>2.16 ± 0.20</td>
</tr>
</tbody>
</table>

Values are means ± SE. $I_\text{sc}$, short-circuit current; $I_\text{K}$, K⁺ current; $K_{1/2}$, apparent equilibrium constant for amine binding; n, Hill coefficient.
Fig. 7. Cytosolic pH responses to apical and basolateral application of ammonia markedly differ. Initial alkalinization in response to apical ammonia (30 mM, ●) relaxes slowly, whereas blunted initial alkalinization in response to basolateral ammonia (30 mM, ○) relaxes rapidly.

Fig. 8. Ammonia inhibition of $I_{sc}$ is independent of ammonia-induced changes in cytosolic pH. Representative curves in A and B labeled $I_{sc}$ depict transepithelial Cl$^-\text{current}$ as a function of time with the voltage clamped at 0 mV and pulsed from 0 to 10 mV at 1-min intervals to provide a record of monolayer resistance change over the course of the experiment. The 2 separate monolayers in A and B were basolaterally stimulated with forskolin (10 mM) at the arrows designated a (t = 14 min), resulting in stimulation of $I_{sc}$ from a resting value of -5 μA to -55 μA within 8 min. Apical treatment with 50 mM NH$_4$Cl at arrow designated b (t = 22 min) results in a 50% decrease in $I_{sc}$ within 28 min. Apical treatment with 50 mM NH$_4$Cl at arrow designated b (t = 22 min) immediately results in a large increase in cytosolic pH that slowly declines with continued apical exposure. B: basolateral treatment with 5 mM NH$_4$Cl at arrow designated b (t = 22 min) results in a 50% decrease in $I_{sc}$ within 16 min. In separate experiments in which polarized and confluent monolayers were mounted to afford isolated apical and basolateral access in a quartz cuvette for fluorescence spectroscopy, representative heavy curves depict the cytosolic pH as a function of time according to the 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) method. Basolateral treatment with 5 mM NH$_4$Cl at arrow designated b (t = 22 min) results in a relatively slow and small increase in cytosolic pH that persists with continued basolateral exposure.
pathways, the pH microenvironment in the vicinity of basolateral K\textsubscript{1} channels may indeed be different from bulk pH.

Ammonia and alkyl amine effects on endosomal pH or membrane recycling do not correlate with inhibitory action. The ability to alter endosomal pH also does not correlate with the ability to inhibit secretion. Figure 10, A–C, depicts the change in the H\textsuperscript{+} concentration (Δ[H\textsuperscript{+}]) of a broad population of endosomal compartments in suspended T84 cells as a function of time after acute treatment with ammonia (1), propylamine (4), and octylamine (9), respectively. The three suspensions were treated separately with the concentrations of amines 1, 4, and 9 required to suppress Cl\textsuperscript{−} secretion by 50% (50\% I\textsubscript{sc}), but the endosomal [H\textsuperscript{+}] responses differ dramatically. All amines initially alkalinize endosomes, but the initial Δ[H\textsuperscript{+}] for ammonia (1) and octylamine (9) is about −0.3 mM and that for propylamine (4) is about −0.9 mM. Furthermore, prolonged incubation with amine 4 alkalinizes endosomal compartments, whereas prolonged incubation with amine 9 acidifies endosomal compartments. Maximal effects of the amines on Cl\textsuperscript{−} secretion are typically reached within 10 min of basolateral exposure and persist for at least 24 h. Because our dose-response measurements reflect a routine 30-min preincubation with amine, our 50\% I\textsubscript{sc} for amines 1, 4, and 9 correspond to a time point at which the effects of the amines on endosomal pH are more pronounced than those on cytosolic pH. Thus neither endosomal nor cytosolic pH correlates with I\textsubscript{sc} change.

Finally, fluid-phase endocytosis was estimated by 6-min uptake of FITC-dextran by T84 monolayers grown on plastic and permeable supports. In our hands, the cAMP agent forskolin slightly inhibits endocytosis (70 ± 10\% relative to control in the absence of forskolin; n = 6) in monolayers grown on plastic, a result similar to that obtained by others (4, 5, 39). Forskolin slightly inhibits apical (80 ± 10\%) but not basolateral (110 ± 16\%) endocytosis in filter-grown cells (n = 12 for each). Ammonia (10 mM) has no effect (96 ± 10\% relative to control in the absence of ammonia; n = 3) on the cAMP-dependent slight inhibition of FITC-dextran uptake in monolayers grown on plastic. Examination of apical and basolateral endocytosis using monolayers grown on permeable supports showed that preincubation (30 min) with ammonia (10 mM) has little or no effect (85 ± 10\% and 92 ± 8\%, respectively, relative to control in the absence of ammonia) on FITC-dextran uptake used as a method to estimate pinocytotic uptake.

DISCUSSION

The assumption that biological effects of ammonia and alkyl amines are exerted via effects on cytosolic
and/or endosomal pH is firmly entrenched and only occasionally questioned. Indeed, these agents are widely used as experimental probes for just this purpose. Our experiments clearly indicate that the mechanism by which ammonia inhibits transepithelial Cl⁻ secretion is independent of its ability to alkalinize the bulk cytoplasm or raise the pH of acidic endosomal compartments. Structure-function and kinetic analyses using a coherent series of amine probes provide direct evidence to support our recent speculation that the target of ammonia action is instead the basolateral K⁺ channel. One is sensitive to the well-known K⁺ channel blocker Ba²⁺, whereas the other is Ca²⁺ dependent but insensitive to Ba²⁺. These K⁺ channels are targets of drug development against secretory diarrheal disorders. Indeed, clotrimazole (41) may act by a mechanism similar to the one we propose here. In data not shown, we found that the small hydrophilic amines 1–3 do not block Ca²⁺-regulated (carbachol-stimulated and Ba²⁺-insensitive) basolateral K⁺ conductance in the apically permeabilized system or transepithelial Cl⁻ secretion in intact monolayers within our dose ranges. The lipophilic amines 4–9, however, do block these Ca²⁺-regulated processes at concentrations effective in blocking resting K⁺ conductance, and inhibitory ability increases with lipophilicity.

Although a number of quartenary ammonium compounds are known to inhibit certain K⁺ channels (21), the effects of ammonia and monoamines have not been defined in most systems. Indeed, a number of K⁺ channels are known to conduct NH₄⁺ quite readily, usually with ~30% or less efficiency (21). None is known explicitly to be competitively blocked by NH₄⁺, but there is a recent suggestion of such NH₄⁺ block in the thick ascending limb of Henle's loop in rat kidney (2, 26, 42). The naturally occurring cystolic polyamines spermine, spermidine, putrescine, and cadaverine may serve as physiological blockers of inward rectifier K⁺ channels (15, 17, 19, 29).

The use of structure-function analysis with the well-defined amine set allows us to speculate on the mechanism of ammonia and primary alkyl amine K⁺ channel block. We propose that the discontinuity at log P = 0 in Figs. 6 and 7 reflects the two different routes by which the two classes of amines reach their target K⁺ channels (Fig. 11). The relatively small hydrophilic amines 1–3 reach their targets by the aqueous path in which they are preferentially solubilized. Inhibitory ability of amines 1 > 2 > 3 shows that the smallest and most water-soluble of the three (NH₄⁺) has the highest probability of occupying the channel site normally occupied by K⁺. As ion size first increases by the volume of a methyl group (2) and then by an ethyl group (3), the ability to occupy the site intended for K⁺ decreases as does the ability to block secretion. In contrast, the large hydrophobic amines 4–9 reach their target by the hydrophobic pathway in which they are preferentially soluble. The concentration of unprotonated amine in the membrane is directly related to its partition coefficient P. Neutral amine diffuses randomly within the plasma membrane and collides with target K⁺ channels with a probability dictated by amine concentration in the membrane. In some of these collisions, the —NH₂ head will protrude far enough into the aqueous channel such that nitrogen is quickly protonated to give a new NH₃⁺ (ammonium) head that is now hydrophilic. The newly protonated ammonium projects into the aqueous pore so that it interferes with the ability of the pore to accommodate K⁺, and the hydrophobic alkyl tail is solubilized by the lipid membrane interior or by a hydrophobic region of the channel protein. The residence time in this blocking configuration is a function of ammonium aqueous solubility (the same for amines 4–9) and of the lipid solubility of the alkyl tail, which increases with chain length. Inhibitory ability increases with membrane solubility and residence time at the target site, both of which increase with increasingly positive log P. This model is analogous to the Hille model (19, 20) for Na⁺ channel block by local anesthetics.
Two additional observations support the aqueous and nonaqueous pathway proposition. First, apical treatment of T84 monolayers with lipophilic amines 4–9 gives the same $I_{sc}$ values as does the basolateral treatment shown in Fig. 6A, but apical treatment with lipophilic amines 1–3 gives $I_{sc}$ values fivefold higher than those obtained by basolateral treatment. Apical lipophilic amines 4–9 travel to their basolateral targets via the hydrocarbon environment of the lipid bilayer with a facility about equal to that of the same lipophilic amines applied basolaterally. Apical lipophobic amines 1–3 are hampered by their low membrane permeabilities. Second, the dominance of the log P variable is underscored by our basolateral $I_{sc}$ results with the di- and trialkyl amines 10–13. These amines differ markedly from their primary counterparts 2–9 in terms of basicity and steric environment at the nitrogen center. A plot of the $I_{sc}$ values vs. log P for amines 10–13 gives a curve that is nearly superimposable on the curve given by 4–9 in Fig. 6A.

We have speculated on the potential role of ammonia as an endogenous negative regulator of intestinal secretion based on evidence developed in the T84 model, but its effect on secretion in native mammalian intestine is as yet undefined. Solomon et al. (45, 46) recently reported that ammonia inhibits CAMP-dependent secretion in the shark rectal gland preparation. The effect of ammonia on epithelial absorptive processes is to our knowledge largely unexplored. It is interesting to note that in the central nervous system ammonia is known to impair synaptic transmission and neurotransmitter release (16, 40, 47). Its impact on neurohormonal regulatory events in the gut is unknown; the effects of ammonia on integrated intestinal function are thus likely to be complex. Recognition that NH$_3$ can block certain K$^+$ channels may well encourage re-evaluation of ammonia’s role in other biological systems, particularly in those instances in which it has been assumed that ammonia’s action is due to effects on cytoplasmic and endosomal pH.

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