AMMONIUM CHLORIDE INHIBITS CHLORIDE SECRETION IN THE RECTAL GLAND OF SQUALUS ACA[illegible]IAS

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Recent studies in intestinal and renal epithelia have indicated that NH_4 can substitute for K in a variety of membrane transport processes including Na, K, 2Cl cotransport and Na+K ATP hydrolysis. In T84 cells, derived from chloride secreting intestinal crypt cells, NH_4 inhibits short circuit current stimulated by cAMP agonists. The following studies were performed to determine the effect of NH_4 on chloride secretion in the rectal gland, a tissue analagous to the intestinal crypt cell.

Rectal glands were perfused with shark Ringer's solution as previously described (Solomon et al., Am. J. Physiol.262: R707, 1992). Nine collection periods of 10 minutes each were obtained during constant stimulation of chloride secretion by theophylline, 2.5×10^{-4} M. After three baseline collections, the perfusate was switched to one that had ammonium chloride, 1, 5, or 10 mM, substituted equimolar for sodium chloride. After three collections during exposure to the ammonium chloride Ringer's, the perfusate was again switched back to the original shark's Ringer's without ammonium chloride. As seen in Figure 1, addition of ammonium chloride (5 mM) to the perfusate produced a statistically significant and reversible inhibition of chloride secretion.

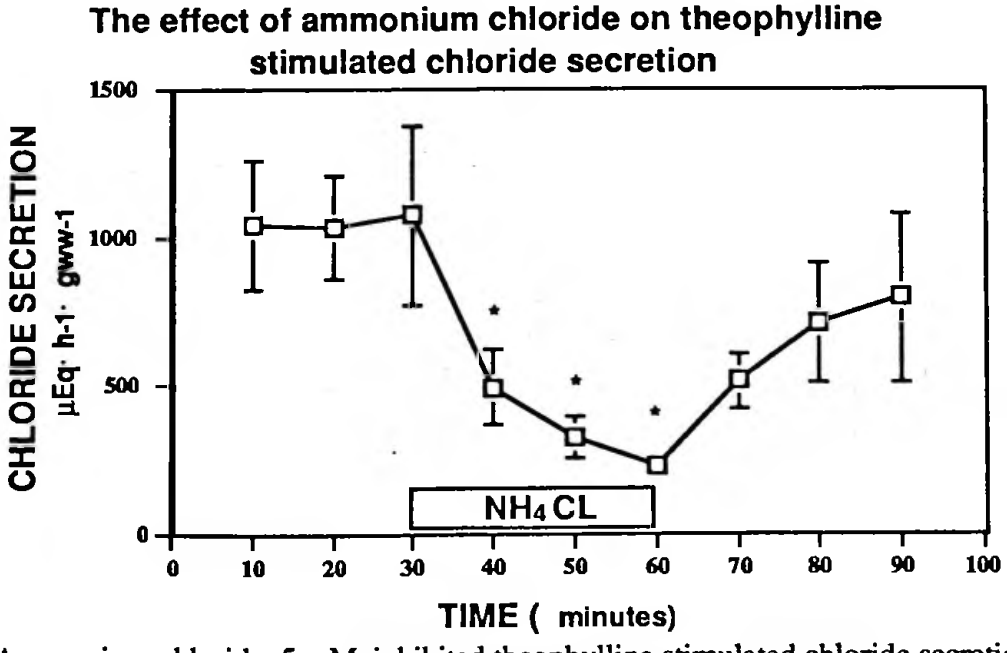

Figure 1.Ammonium chloride, 5 mM, inhibited theophylline stimulated chloride secretion within 10 minutes of exposure. The effect was reversible with removal of ammonium chloride from the perfusate. * $p < .05$ by paired t test compared to the value at 30 minutes.

Rectal glands stimulated by theophylline were exposed to either 1, 5, or 10 mM NH_4Cl (Figure 2). Total chloride secretion was inhibited by 59% at 1mM NH_4, while at 5 and 10 mM NH_4, 73% and 71% of chloride secretion respectively was inhibited. At all concentrations, recovery of chloride secretion occurred rapidly following cessation of exposure to NH_4Cl. The inhibitory effect of 1 mM NH_4Cl was not statistically different from that of 5 mM or 10 mM NH_4Cl.

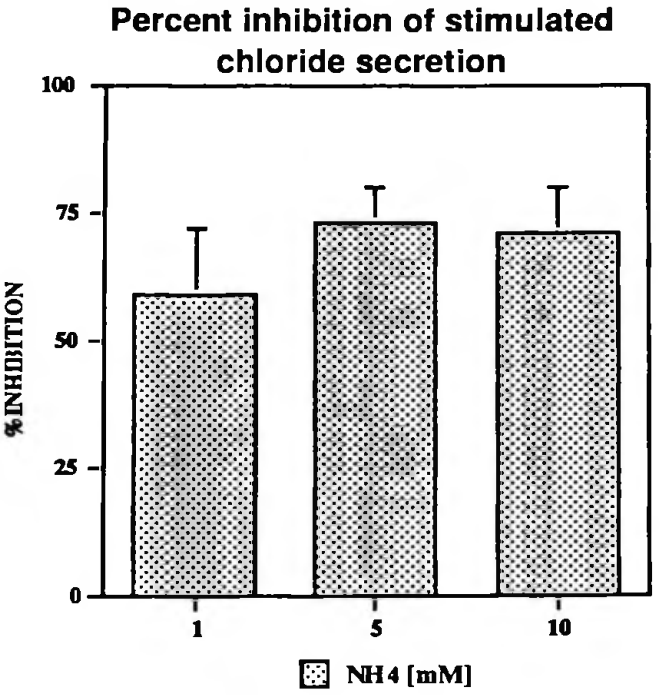

Figure 2. Ammonium chloride inhibits theophylline stimulated chloride secretion.

NH_4Cl (5 mM) also inhibited chloride secretion stimulated by forskolin and genistein (Figure 3). The degree of inhibition was similar to that observed under stimulation with theophylline. Recovery following removal of the NH_4Cl was again rapid and complete.

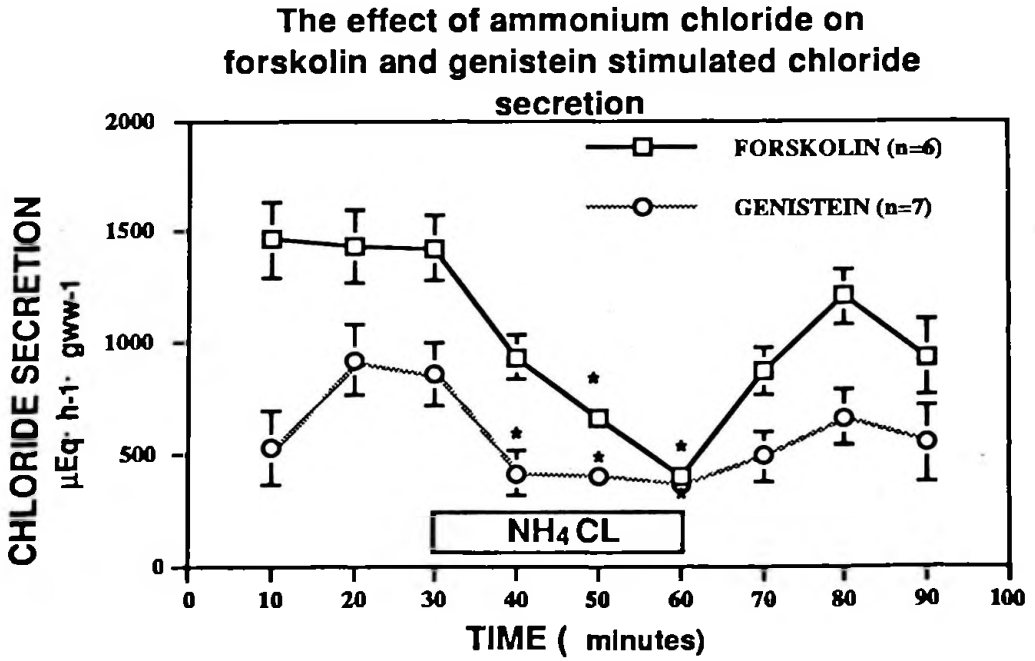

Figure 3. Ammonium chloride, 5 mM, inhibited both forskolin and genistein stimulated chloride secretion within 10 minutes of exposure. The effect was reversible with removal of ammonium chloride from the perfusate. * $p < .05$ by paired t test compared to the value at 30 minutes.

NH_4Cl also inhibited short circuit current in primary cultures of rectal gland cells grown to confluence on collagen supports and mounted in Ussing chambers. Ion transport was stimulated with C-type natriuretic peptide (10^{-7}M) and NH_4Cl (5 mM) applied to the apical surface. Short circuit current was inhibited 50 ± 11% (n=3; data not shown).

NH_4Cl is a weak acid and can affect intracellular pH either as a result of diffusion of NH_3 or transport of NH_4 ions into the cell. We determined the effect of NH_4Cl incubation on intracellular pH of freshly prepared rectal gland tubules loaded with the pH sensitive fluorescent dye, 2'-7'-bis(carboxyethyl)-5(6)-carboxyfluorescein, BCECF. Fluorescence was monitored in a dual beam spectrophotometer and the ratio of fluorescence at 439 and 505 nm calculated. The exposure to either 5 or 10 mM NH_4Cl led to transient alkalinization but pHi rapidly returned to basal levels and remained stable for at least 10 minutes (Figure 4). Nigericin that allows equilibrium between the intracellular and extracellular pH was used as a positive control for alkalinization. The transient alkalinization following exposure to NH_4Cl presumably results from the diffusion of NH_3 into the cells, acquisition of a H^+ to form NH_4^+ resulting in an increase intracellular pH.

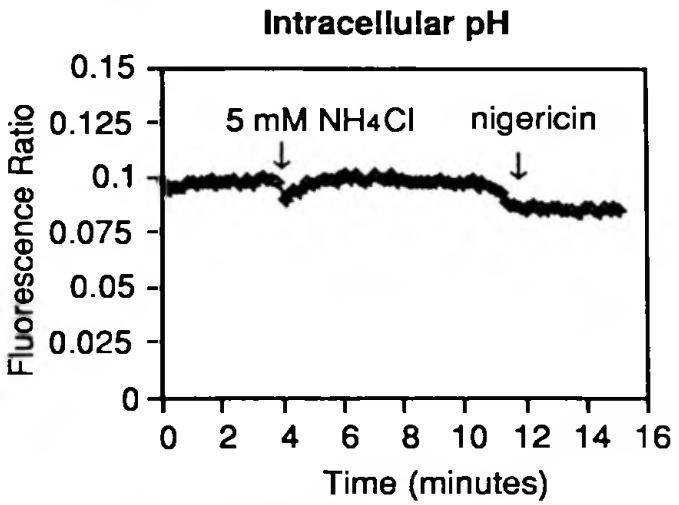

Figure 4. Intracellular pH measured with the fluorescent dye BCECF was not affected by exposure of the cells to ammonium chloride.

These preliminary observations provide evidence that NH_4Cl inhibits chloride transport. The experimental design indicates that it is the NH_4 ion which produces these effects. The results confirm the observations of Prasad et al. in T84 cells (J. Clin. Invest. 96:2142,1995). The inhibition does not appear to be a consequence of an effect of NH_4 on intracellular pH as this remained unchanged during the period of inhibition of chloride secretion. Inhibition of both cAMP and cGMP mediated stimulation of chloride secretion suggests that the effect of NH_4^+ occurs at a distal site in the intracellular signalling cascade leading to enhanced chloride secretion, perhaps at the chloride channel itself.

The physiologic importance of these observations is unclear. In elasmobranchs, a reduced capacity (relative to mammals) to synthesize urea from NH_3 results in a 10 fold higher concentration of blood NH_4 than that found in mammals (Goldstein, L. personal communication). Arterial blood levels are in the order of 0.2-0.4 mM. Future studies will address whether there is an effect of such levels on chloride secretion by the rectal gland and the mechanism of the inhibitory effects reported herein.

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