FURTHER STUDIES ON THE INHIBITION OF CHLORIDE SECRETION BY AMMONIUM IN THE RECTAL GLAND OF *SQUALUS ACANTHIAS*

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We previously reported that ammonium chloride produced reversible inhibition of cyclic nucleotide stimulated chloride secretion by the rectal gland (Solomon et al., Bull. MDIBL 35: 37-9, 1996). The mechanism of the inhibition is unknown although there is speculation that inhibition may involve blockade of a potassium conductance. Rubidium loss from T84 cells exposed to ammonium chloride is inhibited supporting this hypothesis (Matthews - personal communication). In this report, we explored the lower end of the dose response relationship between ammonium and chloride secretion. We then used ethylamine and n-octylamine to explore the effects of organic substitution and lipophilicity. The results with these organic compounds were compared to barium chloride and tetraethylammonium (TEA), compounds known to block some potassium conductance pathways.

Rectal glands were perfused as previously described (Silva et al., Methods Enzymol. Vol 192:754-66, 1990) with shark Ringer's solution containing 0.25 mM theophylline to induce stimulation of chloride secretion. Under these conditions, chloride secretion remains high for the duration of the infusion. A total of nine collection periods of 10 minutes each were obtained. After three baseline collections, the perfusate was switched to one that contained the test compound. After three more collections during perfusion with the test compound, the perfusate was switched back to the original shark Ringer's solution without the test compound to assess reversibility. The percent inhibition was calculated as the 100% minus the ratio of chloride secretion during the last period of exposure to the test compound and the chloride secretion during the last control period immediately prior to exposure to the test compound.

Ammonium chloride 1.0 and 2.5 mM significantly inhibited stimulated chloride secretion by 31±6% and 45±8% respectively ($p < .05$ by paired t test for both concentrations). The dose response curve between 1.0 and 10 mM (including data from 1995) is depicted in Figure 1. A maximal inhibitory effect is present at 5 mM ammonium chloride. Significant inhibition is present at the 1 mM level which is reversible upon removal of the ammonium chloride from the perfusate.

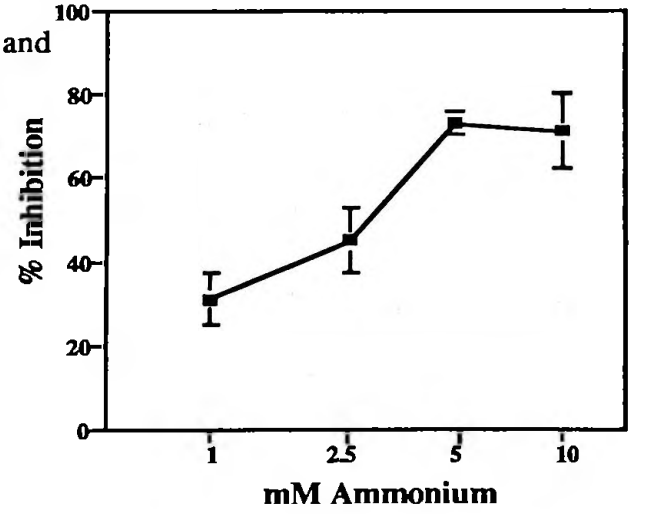

Figure 1.

Ethylamine and n-octylamine were studied to assess whether organic substituted ammonium was equally effective in producing inhibition. The use of these compounds was supported by the recognized ability of tetraethylammonium to interfere with potassium conductances in some cell types.

Both ethylamine (1 mM) and n-octylamine (0.5 and 1 mM) produced inhibition of stimulated chloride secretion (Figure 2). N-octylamine appeared to be more potent than ammonium chloride although a full dose response relationship was not explored. Since n-octylamine is more lipophylic compared to ammonium, the mechanism of action may require entry of the compound into the membrane of the cell.

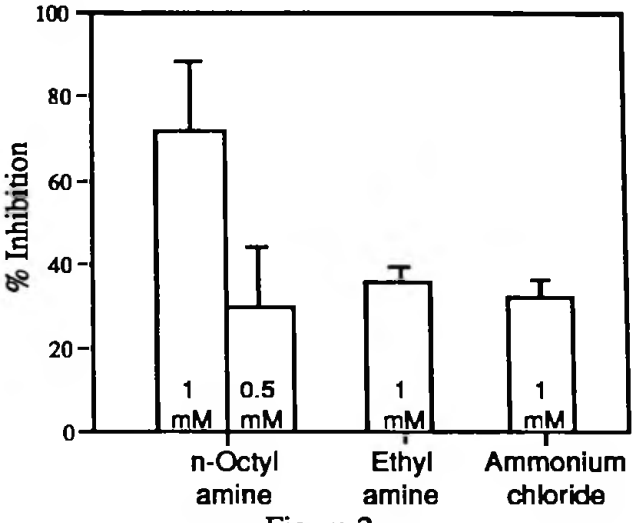

Figure 2.

The mechanism of the inhibitory action of these compounds is unknown. One speculation is that ammonium interferes with potassium conductance. This is supported by the greater inhibitory effect of ammonium when applied to the basolateral compared to the apical side of cultured T84 cells, an intestinal epithelium which actively transports chloride in response to cyclic nucleotides (Prasad et al., J. Clin. Invest. 96:2142-51, 1995).

Experiments were conducted with barium chloride (0.5 mM) and tetraethylammonium (5 mM) to establish the effects of known inhibitors of potassium conductance pathways. As reported by a number of other investigators, barium chloride inhibited chloride secretions in this tissue to 69±3%. In contrast, tetraethylammonium 5 mM was without effect (Figure 3). The effects of n-octylamine 0.5 mM were of a similar magnitude to those of barium chloride.

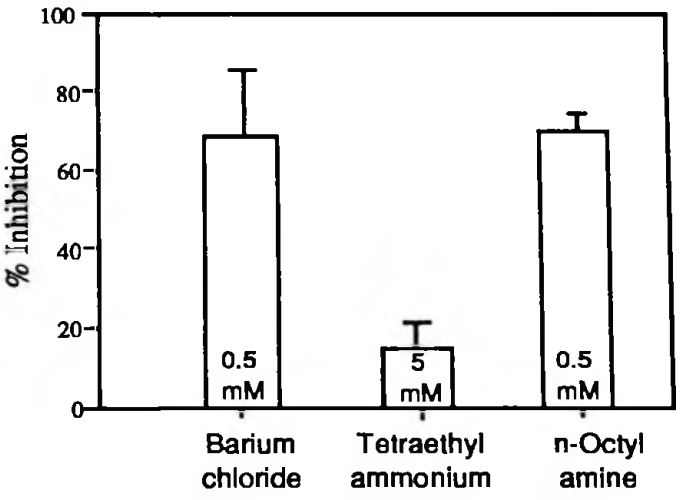

Figure 3.

The present experiments confirm the initial observations of an inhibitory effect of ammonium and extend this inhibitory effect of organic compounds of ammonium with high lipophylicity. This supports the speculation that the inhibitory effect is occurring at the level of the cell membrane. Furthermore, n-octylamine does not dissociate into ionic species, unlike ammonium chloride, and therefore does not affect intracellular pH. In T84 cells, the inhibitory effect of ammonium chloride is specific for cyclic nucleotide stimulated chloride secretion suggesting that it is not mediated through a generalized toxic effect.

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