

Effects of amiloride and furosemide on histamine release from human leukocytes induced by anti-IgE antibody

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Inhalation of the "loop" diuretic furosemide inhibits both early- and late-phase bronchospasm in response to allergen bronchoprovocation in patients with allergic asthma.¹⁻³ Inhibition of histamine release from airway mast cells has been proposed as a possible mechanism.

Amiloride, an inhibitor of the sodium/proton (Na^+/H^+) antiport system, has previously been shown to inhibit the contraction of isolated, passively sensitized guinea pig trachea smooth muscle in response to antigen challenge, and even appears to interfere with sensitization itself.⁴ This effect is believed to be a result of alteration of sodium influx at the level of the cell membrane. In addition to its effects on smooth muscle, amiloride and its analogs have been shown to inhibit tritiated serotonin release from dinitrophenol sensitized rat basophilic leukemia cells (RBL-2H3) in response to antigen challenge. This effect is apparently mediated through specific inhibition of a sodium-dependent calcium influx pathway.⁵

In this study we investigated the effects of varying concentrations of amiloride and furosemide on histamine release from human leukocytes induced by anti-IgE antibody to determine if a concentration-dependent inhibition occurs that may explain, in part, the observed effects on asthma and offer insight into the mechanism of histamine release.

The histamine release assay was performed with use of a histamine radioimmunoassay kit purchased from AMAC, Inc., Westbrook, Maine. The four subjects for this study had a history of either asthma or allergic rhinitis (or both) and multiple positive skin

tests demonstrated by skin puncture or intradermal testing in our allergy clinic. All gave informed consent. Venous blood (10 to 15 ml) was collected in heparinized glass tubes, divided into 200 mm^3 aliquots, and immediately placed on ice. After preincubation of the aliquots of blood with amiloride (at concentrations of 2 mmol/L , 20 $\mu\text{mol/L}$, and 200 $\mu\text{mol/L}$), or furosemide (at concentrations of 30 mmol/L and 300 $\mu\text{mol/L}$), the histamine release assay was performed in quadruplicate with use of sheep antihuman IgE antibody, according to the directions in the histamine radioimmunoassay kit. Nanomolar concentrations of histamine were calculated according to the dilutions made. Statistical analysis was performed on the histamine concentrations with use of a Student's *t* test. Significance was determined as *p* values of 0.05 or less. No vehicle (solvent) effects were observed on either histamine release or the radioimmunoassay.

The percent of maximal histamine release after *in vitro* amiloride and furosemide incubation is shown in Fig. 1. Histamine release in the control specimens (untreated with anti-IgE) was negligible in amount and was unaffected by amiloride or furosemide. Amiloride pretreatment resulted in statistically significant inhibition of histamine release in all four subjects at a concentration of 2 mmol/L and in three subjects at a concentration of 20 $\mu\text{mol/L}$. Furosemide pretreatment did not result in significant inhibition even at the maximal concentration of 30 mmol/L and may have actually stimulated a low level of histamine release. No statistically significant inhibitory effect on release induced by calcium ionophore A23187 (dissolved in ethanol) was seen with either agent (data not shown). Vehicle controls without the agent of interest produced no effect. Neither diuretic caused release when incubated alone.

These data suggest that amiloride inhibits histamine release from human basophils, and this effect appears to be dependent on concentration. Furosemide did not demonstrate a similar effect and may even result in potentiation of release. The observed inhibition of

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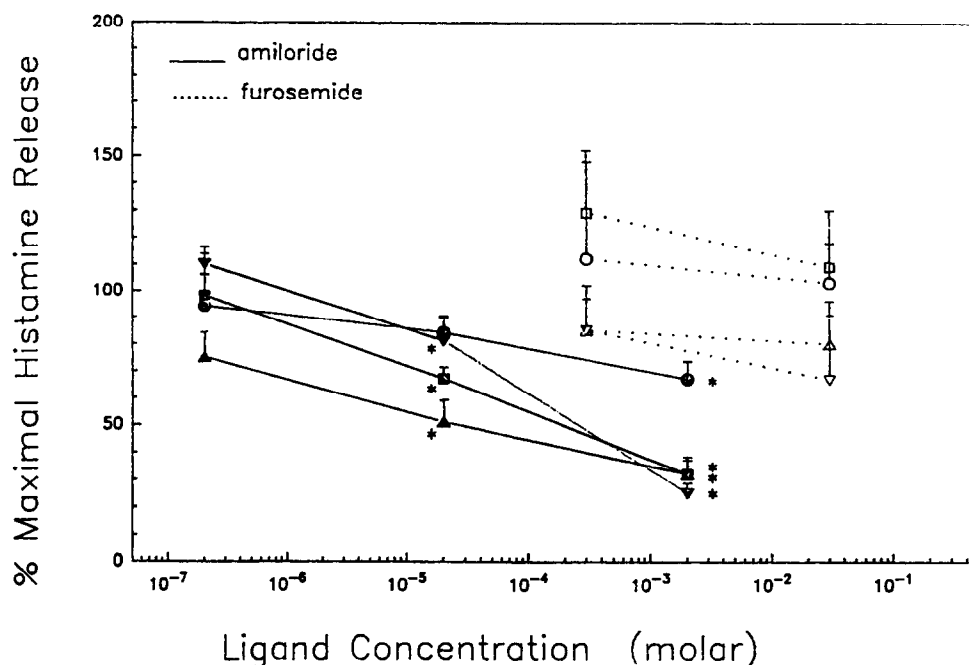


FIG. 1. Effects of amiloride and furosemide on histamine release. Maximal histamine release seen when neither diuretic was added and the cells were incubated with the anti-IgE antibody assigned a value of 100%. This corresponds to nanomolar concentrations of 46.78 ± 7.32 for subject 1, 46.85 ± 8.01 for subject 2, 42.20 ± 8.17 for subject 3, and 22.80 ± 1.16 for subject 4. Increasing concentrations of amiloride resulted in statistically significant inhibition of histamine release in all four subjects at a concentration of 2 mmol/L and in three of the four subjects at a concentration of 20 μ mol/L. Furosemide showed no inhibition of histamine release at the concentrations tested.

histamine release by amiloride was statistically significant in all subjects at a concentration of 2 mmol/L.

The specificity of amiloride as a Na^+/H^+ antiporter inhibitor and the concentration dependence of the inhibitory effect observed suggests that histamine release in human basophils, in response to antigen cross-linking of surface-bound IgE, may involve a calcium influx pathway dependent on sodium. The failure of amiloride to inhibit release stimulated by A23187 suggests that the calcium ionophore bypasses the sodium-dependent calcium pathway. Since furosemide does not affect the Na^+/H^+ antiport, inhibition through this mechanism would not be anticipated. Use of both diuretics, as well as analogs of amiloride, which possess differential specificities for a number of ion transport systems, will facilitate the analysis of the role of ion influxes in histamine release. Amiloride-induced inhibition of sensitized guinea pig tracheal smooth muscle in response to antigen challenge has been reported.³ Another report that describes furosemide inhibition of histamine release from lung fragments suggests that inhibition of histamine release in lung may involve another mechanism or a different dose re-

sponse curve than is found with basophils.⁶ The therapeutic value of amiloride (and other analogs with potent inhibitory effects in the Na^+/H^+ antiport⁵) deserves further study.

REFERENCES

1. Bianco S, Pieroni M, Refini M, et al. Protective effect of inhaled furosemide on allergen-induced early and late asthmatic reactions. *N Engl J Med* 1989;321:1069-73.
2. Bianco S, Pieroni M, Refini M, et al. Inhaled furosemide prevents allergen-induced bronchoconstriction in atopic asthmatics. *Am Rev Respir Dis* 1988;137(suppl):27.
3. Robuschi M, Pieroni M, Refini M, et al. Prevention of antigen-induced early obstructive reaction by inhaled furosemide in (atopic) subjects with asthma and (actively sensitized) guinea pigs. *J ALLERGY CLIN IMMUNOL* 1990;85:10-6.
4. Souhrada M, Souhrada MH, Souhrada JF. The inhibition of sodium influx attenuates airway response to a specific antigen challenge. *Br J Pharmacol* 1988;93:884-92.
5. Stump RF, Oliver JM, Crasgoe EJ, et al. The control of mediator release from RBL-2H3 cells: roles for Ca^{2+} , Na^+ , and Protein Kinase C. *J Immunol* 1987;139:881-6.
6. Anderson DS, He W, Temple DM. Inhibition of furosemide of inflammatory mediators from lung fragments. *N Engl J Med* 1991;324:131.