therapy. Responses returned to control levels when medication was discontinued. However, there was a suggestion that the recovery period might be somewhat longer in asthmatic adults than in children.

To determine whether the effects of oral sympathomimetic agents on leukocyte cyclic AMP responses might differ in normal and asthmatic subjects, a comparison was made of the effects of short-term treatment with ephedrine in the two groups. All subjects were treated with ephedrine 25 mg four times daily for seven days; asthmatic patients were also given one week of placebo treatment in randomized order. Results obtained during the control and placebo periods again demonstrated that when asthmatic patients were not receiving treatment with adrenergic medications, the leukocyte cyclic AMP measurements were similar to those of normal subjects. Treatment with ephedrine for seven days was associated with reduction in the leukocyte cyclic AMP responses to adrenergic stimulation in both groups. However, in normal subjects the changes in response barely achieved significance (P < .05 by comparison of paired data), whereas in asthmatic patients, the changes were highly significant (P < .001). Comparison of the mean results in the two groups also demonstrated that the reduction of response after identical therapy with ephedrine was significantly greater in asthmatic subjects (P < .01).

In the above study after collection of the initial specimens, subjects were given 200 μg of epinephrine by subcutaneous injection and a second sample was obtained two hours later. In both groups, leukocytes obtained after treatment with epinephrine were virtually unresponsive to further adrenergic stimulation in vitro. There were no differences in the responses of specimens obtained during the control and ephedrine periods. However, in asthmatic subjects, the reduction of response after seven days of treatment with ephedrine was comparable to that observed after administration of epinephrine (J Cyclic Nuc Res, 3:439, 1977).

The results of our studies indicate that the altered leukocyte responses which have been observed in asthmatic subjects are largely due to the effects of recent therapy with adrenergic medications rather than the underlying disease. The similarity of results after treatment with several different medications suggests that exposure to any beta-adrenergic agonist is followed by suppression of the leukocyte responses to further adrenergic stimulation. The change in response can be demonstrated in both normal and asthmatic subjects within two hours after administration of an intense adrenergic stimulus and persists for a relatively long time. After administration of smaller amounts of medication the suppression of leukocyte response appears to be greater in asthmatic patients than in normal subjects, suggesting a possible difference in the susceptibility of the two groups to suppression or in the time required for recovery of normal response. This may indicate a difference in the number or availability of cellular beta-adrenergic receptors in the two groups.

Comparisons of the Effects of Fenoterol on PCA and Histamine Skin Test in Rats

Robert G. Townley, M.D. and Kenji Mano, M.D.

The purpose of these studies was to compare the effect of fenoterol with other antiallergy drugs such as cromolyn sodium, dephenhydramine (Benadryl) and two phosphodiesterase inhibitors, imidazolidinone and aminophylline on the inhibition of the allergic reaction. The PCA, or passive cutaneous anaphylaxis, response in rats was the model used to compare these agents. When antigen combined with Evans blue dye is given intravenously to sensitized rats, there is an extravasation of the dye due to release of mediators, and when similar rats are pretreated with different doses of cromolyn, you can markedly inhibit that response.

A mast cell that had been pretreated with cromolyn and then challenged with the specific antigen remains intact and there is no loss of granules or mediators.

Control rats show the release of the granules with their mediators in this system. We evaluated the potent phosphodiesterase inhibitor imidazolidinone. The agent is several hundred times more potent than theophylline in causing relaxation of isolated human or guinea pig tracheal smooth muscle. We also showed that both imidazolidinone and cromolyn are quite capable of inhibiting mediator release. However, these agents do this by different mechanisms. Cromolyn sodium has no effect on cyclic AMP in the mast cell or tracheal smooth muscle and does not cause relaxation of respiratory smooth muscle. Imidazolidinone is an extremely potent phosphodiesterase inhibitor and its effect on inhibiting mediator release is through that mechanism.

A comparison using much lower doses of the two drugs shows less inhibition of the PCA reaction. At 1 mg/ml the phosphodiesterase inhibitor had no significant effect, while cromolyn was still effective. We also measured inhibition of histamine release in vitro, and in this system the phosphodiesterase inhibitor, imidazolidinone, was more effective than cromolyn sodium. Theophylline and cromolyn were about equally effective in this in vitro system.

We then began to compare the effect of the beta₂ agonist, fenoterol, on the PCA response. In the control rats, the PCA response is a 30 mm diameter wheal which decreases to about a 10 mm wheal as we serially dilute the antiserum. Fenoterol works in a dose-dependent manner, and if we use a large dose of fenoterol, far more than one would ever use clinically (10 mg/kg IV), there is a more marked suppression of the PCA response. However, even with doses down to 10 mg/kg there is still inhibition of the PCA, particularly at the more dilute serum concentration, where there is less mediator release. The differences were much less striking when we had a greater antigen-antibody reaction and more medi-
results were obtained when we used the four-hour PCA instead of the 72-hour FCA. Again, with increasing doses of fenoterol, from 1 μg to 10 mg there is further inhibition of the PCA responses.

We then compared fenoterol with diphenhydramine (Benadryl) and aminophylline. We used two doses of fenoterol, 10 micrograms or 10 milligrams per kilogram. At doses of 10 micrograms per kilogram, both fenoterol and Benadryl showed approximately the same effect on the PCA. Aminophylline was considerably less effective. To my knowledge, antihistamines of the H1 class do not inhibit mediator release. The inhibitory effect is rather direct where the dose of histamine is released, thus preventing the increased permeability.

Using the same doses of antihistamine, we compared inhibition of the PCA response with inhibition of the direct histamine skin test response. If we have low doses of histamine then even low doses of antihistamine are completely effective in inhibiting the wheal response. But as Dr. Lichtenstein has pointed out, the concentration of an antihistamine in vivo is probably around 10^-4 where the dose of histamine in skin tissue may be as high as 10^-3. Thus, where we inject larger concentrations of histamine intradermally, we are getting, percentage-wise, less inhibition of the skin response, even with fairly high doses of antihistamine injected systemically. We then studied the effect of fenoterol on direct histamine skin responses to compare them with the PCA responses. Again, there is a parallel inhibition of the direct histamine response as we gradually increase the dose of fenoterol from 1 μg/kg up to 10 mg/kg. If a very small amount of histamine is used and a large amount of the β2 agonist, we observed partial inhibition of the histamine response. Thus, from doses of 10 or 100 μg/kg or above, there is significant inhibition of this direct effect of histamine.

We then compared fenoterol with the antihistamine and with aminophylline in the same system in inhibiting the direct histamine response. Here, the antihistamine Benadryl is the most effective, particularly, at low doses of histamine. Fenoterol was the next most effective and aminophylline had the least effect. Aminophylline was not very effective in this system.

We attempted to combine the phosphodiesterase inhibitor aminophylline with fenoterol to see if we could further inhibit the PCA response. The combination of the two agents was no greater than fenoterol alone in this system.

We then compared the inhibition by fenoterol on PCA versus its inhibition of the direct histamine skin reaction. The greatest inhibition was on the PCA reaction. Although it inhibited both reactions, it had less effect on the direct histamine response than it did on the PCA. This is probably because fenoterol is exerting two effects: 1) by increasing cyclic AMP in the mast cell it inhibits mediator release, 2) by its beta adrenergic property as a physiologic antagonist to histamine and in that respect, inhibit the direct effect of histamine.

I would like to briefly discuss the above findings with some of the things Dr. Permutt has discussed. If we challenge asthmatics with an allergen, there is a marked decrease in FEV1. If the asthmatics are given cromolyn sodium there is a marked inhibition of that response, but obviously not a complete inhibition. Cromolyn had no effect in the asthmatic on the methacholine or on the histamine inhalation airway response. So, in this system where you use an allergen challenge, you can significantly protect against the allergen response with an agent such as cromolyn sodium that has no effect on cyclic AMP, by inhibiting mediator release. In addition, it has no bronchodilating effect or protection on the smooth muscle constricting effect of histamine or methacholine.

When we looked at both the immediate response and the delayed response in terms of symptoms, cromolyn was able to inhibit both the immediate and delayed symptoms resulting from the allergen inhalation challenges. Cortisone will inhibit the delayed but not the immediate response, whereas beta adrenergic agonists will inhibit the immediate but not the delayed.

Subsequently we compared the sensitivity of asthmatics to methacholine and histamine. We then compared the effect of an anticholinergic agent with a beta adrenergic agent against both methacholine and histamine. Finally, we attempted to determine the site of action, large or small airways, of methacholine and histamine and also in what airways the cholinergic antagonists and the beta agonists are working.

Eight asthmatics who had at least a 20 percent improvement in the FEV1, with isoproterenol were studied in a randomized double-blind cross-over manner. They were challenged with either methacholine or histamine following placebo, the anticholinergic agent (SCH-1000) or metaproterenol. Methacholine or histamine was administered using doubling concentrations and the effect on the FEV1 was measured. In the control response, a mean dose of 0.6 mg of methacholine caused a 37 percent decrease in the FEV1. The beta agonist had a bronchodilating effect, and allowed the individuals to take considerably more methacholine. However, the anticholinergic agent, which produced less of a bronchodilating effect compared to the beta agonist, was much more effective in inhibiting the methacholine response. There was no significant difference in the average response to methacholine, or histamine, in these same subjects. Although the anticholinergic agent was more effective in blocking the methacholine response than the beta2 agonist, there was no protection by the anticholinergic agent against histamine. The beta adrenergic agonist was equally protective against both methacholine and histamine. There was also no difference between methacholine and the histamine on the small airways as determined by the MEFV. Again, there was marked protection of the small airways by the anticholinergic agent against methacholine, but no protection against histamine. Again, the beta agonist is equally protective against both metha-
choline and histamine effects on the small airways.

The effect on the large airways, as measured by the peak expiratory flow rate, again showed no difference between methacholine and histamine. The anticholinergic agent was very effective against methacholine. However, in contrast to the small airways, there was slight protection by the anticholinergic against histamine in the large airways.

**Effect of Beta-Adrenergic Agents on Skin Test Responses and Bronchial Challenge Responses**

_Sheeldon L. Spector, M.D., F.C.C.P._

_In vitro_ models have shown that both beta2 adrenergic agonists and theophylline derivatives cause a net increase in cyclic adenosine monophosphate (cyclic AMP) and inhibit the release of mediators such as histamine from mast cells and basophils. Blocking cyclic guanosine monophosphate (cyclic GMP) also inhibits mediator release (Lichtenstein LM, et al: Science 161: 902, 1968; Orange RF, et al: J Exp Med 134:1365, 1971). The following human studies represent attempts to provide in_vivo correlates to these in_vitro models.

The first study involves 12 patients with allergic rhinitis and reagin-mediated reactivity, as defined by a positive skin test to at least five different antigens at either 10-3, 10-4, or 10-5 weight per volume with a 6 mm wheal greater than the diluent control (Spector SL, et al: Med Clin North Am 58:71, 1974). Throughout the study a patient received only three of the six antigens to which he was proven positive, as well as a diluent control and 0.275 mg of histamine phosphate (0.1 mg histamine base). Immediately following the reading of the 20 minute wheal and flare reaction, which was first placed on the left side of the back, loading doses of the following were given: (1) aminophylline 7 mg/kg anhydrous theophylline; (2) fenoterol, 10 mg; (3) placebo. All were administered in a randomized fashion. One and one-half hours later, skin tests were repeated in mirror-image fashion on the opposite side of the back and read 20 minutes later. Skin tests were performed at the same time of day in similar locations on the back to insure consistent skin reactivity.

A special adaptor, which can be used with any tuberculin syringe, was made so that an accurate delivery of the antigen solution could be effectuated with two turns of the adaptor, thereby delivering 0.02 ml of test substance. The wheal and flare diameters of all the reactions were photographed (Ektachrome film). A mm rule was photographed alongside to insure the same enlargement. The slide was then projected onto a screen and magnified four times to facilitate tracing the wheal. The area was then computed using a compensating polar planimeter.

Theophylline blood levels, obtained at the same time the skin tests were read, in 9 of the 12 patients, were between 10 and 20 µg/ml. The other three patients had

![Figure 1. The blocking effect of isoproterenol, SCH1000 (an atropine analogue) and the combination of methacholine and histamine inhalation challenges expressed by changes in standardized concentration increments.](image)

<table>
<thead>
<tr>
<th></th>
<th>Methacholine</th>
<th>Histamine</th>
<th>Methacholine</th>
<th>Histamine</th>
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<td>5.9*</td>
<td>5.8*</td>
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*Represents mean % difference in FEV1 for methacholine and histamine inhalations with and without ephephrine and aminophylline.