Relationship between Beta-adrenergic Binding in Lymphocyte and Severity of Disease in Asthma

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A proposed theory for the development of bronchial asthma suggests the presence of partial or complete blockage of the beta-adrenergic nervous system.¹,² Evidence to support this hypothesis stems from observations that individuals with bronchial asthma have increased sensitivity to beta-adrenergic blocking agents, as well as specific pharmacologic, physiologic and biochemical alterations.³-5 The present investigation was designed to assess lymphocyte beta-adrenergic receptors in asthma employing binding studies using labeled dihydroalprenolol (DHA).⁶-¹¹ In addition, the effects of hydrocortisone on DHA binding was also investigated.

MATERIALS AND METHODS

Individuals studied were ten normal subjects (seven women and three men) with a mean age of 32 years, and 11 stable asthmatic patients (nine women and two men), with a mean age of 39 years. Particular attention was paid to medication taken. In general, patients did not receive or rarely used beta-adrenergic agonists either administered orally or as aerosol, but took theophylline preparations for relief of symptoms. Four asthmatic subjects required 5 to 10 mg of prednisone daily for control of disease. Studies of DHA binding involved a modification of the method reported by Williams et al.¹⁰

RESULTS

In asthmatic subjects, specific DHA binding appeared saturable. For all incubation concentrations of DHA, the specific binding was lower in the asthmatic group when compared to the controls.

Figure 1 demonstrates specific DHA binding at 12 nM concentration. The mean ± standard error for the control group was 391 ± 40 fM/mg protein and 263 ± 35 fM/mg protein for the asthmatic subjects (P < 0.05).

Figure 2 shows specific DHA binding at 11 nM plotted against a measurement of severity of asthma, FEV₁/FVC% and an explanation for variations in specific binding among asthmatic subjects. Those asthmatic subjects with the more severe airway obstruction had lower DHA binding to lymphocyte membrane protein (r=0.93, P < 0.01).

Lymphocytes from normal subjects were divided into two equal aliquots and incubated for 16 hours in phosphate-buffered saline solution with added glucose; 10 nM of hydrocortisone was added to the incubation mixture of one of the aliquots, but not the other. Following incubation, lymphocyte membrane preparations were obtained and binding studies were performed.

Figure 3 shows the results of four studies and demonstrates that specific alprenolol binding is significantly greater in lymphocyte membrane protein preincubated with hydrocortisone. The mean value of specific bound alprenolol for hydrocortisone-incubated lymphocytes was approximately four times greater, ie 491 fM/mg protein compared to 116 fM/mg protein in the aliquots not incubated with hydrocortisone (P < 0.05).

DISCUSSION

The results of the present investigation support the preliminary reports of Kariman and Lefkowitz, who also demonstrated statistically significant lower lymphocyte DHA binding in asthmatic subjects as compared to controls subjects.

The variation in specific DHA binding noted among asthmatic individuals in this investigation and the study by Kariman and Lefkowitz can be explained by differences in disease severity among patients. Those with the most severe disease (as measured by FEV₁/FVC%)

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theophylline preparations were the predominant therapeutic agents used and administration of beta-adrenergic agonists was limited. This is important since there have been in vitro and in vivo studies which have reported receptor inactivation and/or beta-adrenergic unresponsiveness following the use of beta-adrenergic agonists.

The relationship between lower DHA binding and greater severity of disease noted in the present investigation supports findings of Parker and Smith. These investigators reported that there was less increased intracellular cyclic AMP in lymphocytes in response to isoproterenol incubation, in those asthmatic subjects with the more severe and chronic disease.

While the mechanism of action of glucocorticoids in bronchial asthma has long been a subject of controversy, glucocorticoids have been demonstrated to restore or increase responsiveness of tissues and/or cells to catecholamines. The data from the present investigation suggest that this phenomenon may be the result of hydrocortisone causing an increase in the numbers or restoring “sensitivity” to beta-adrenergic receptors available for drug action.

The mechanism to explain differences in DHA binding among asthmatic subjects could include genetic or acquired defects in receptor protein synthesis, receptor protein degradation or change in pools. The clinical significance of the apparent lymphocyte beta-adrenergic receptor abnormality noted in the present investigation needs further study. In patients with bronchial asthma it may reflect a general beta-adrenergic receptor defect. Whether change in receptor activity is dynamic in any individual patient, changing as disease severity varies, needs to be documented. Results from the present investigation suggest that this theory must be given serious consideration.

REFERENCES

4 Reed CE, Cohen M, Enta T: Reduced effect of epinephrine on circulating eosinophils in asthma and after beta-adrenergic blockade or Bordetella pertussis vaccine. J Allergy 46:90-102, 1970

Figure 2. A highly significant positive correlation is shown between specific DHA binding to lymphocyte membrane and 
FEV1/FVC%; r=0.93, P<0.01. Shaded area represents normal values ± 2 SEM.

Figure 3. Labeled DHA binding studies to lymphocyte membrane protein at 13 nM DHA concentration. Lymphocytes were preincubated with (closed circles) or without (open circles) 10 nM hydrocortisone for 14-16 hours prior to binding assays.
10 Williams LT, Snyderman R, Leffkowitz RJ: Identification of B-adrenergic receptor in human lymphocytes by (-)

DISCUSSION

Dr. Turner-Warwick: Did you differentiate your asthmatics in terms of severity of their disease, and did you find any differences between groups in their response to isoproterenol beyond an hour’s time?

Dr. Brooks: That is a good question. We did differentiate asthmatics in terms of severity of their disease. All our patients were stable for a period of months. We assessed severity of disease using clinical and physiologic criteria. We did study one actually ill asthmatic patient. She had decreased alprenolol binding which increased with her clinical improvement during hospitalization. Serial studies on more patients need to be performed in order to determine the duration, magnitude and significance of these changes in alprenolol binding.

Dr. Chick: Were any of your patients recently on beta adrenergic drugs?

Dr. Brooks: This is important since the use of beta adrenergic agonist drugs has been shown to depress in vitro beta-receptor binding. We selected patients who had either never taken beta adrenergic agonist agents or who had not received them for weeks to months prior to study.

Dr. Oren: Is there a dose-response relationship in the effect of glucocorticoid on binding, and is it different for different patients, depending on the severity of illness?

Dr. Brooks: In vitro studies suggest that this may be true, but this is as yet unstudied.

In vitro Studies on the Mechanism of Respiratory Virus-induced Asthma*

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The mechanism of virus-provoked episodes of asthma is not established. One of several plausible hypotheses is an impairment by virus or some product of virus-infected cells of homeostatic mechanisms that maintain normal bronchial caliber. Release of granulocyte (PMN) lysosomal enzyme, beta-glucuronidase, follows incubation with complement activated zymosan. In asthma, isoproterenol (ISO) and histamine (HIS) inhibition of enzyme release is impaired while prostaglandin E1 (PGE) response is normal (Am Rev Respir Dis 115:783, 1977; J Clin Invest 59:1080, 1977). During respiratory infection-provoked asthma, the response to ISO is further impaired. A similar, but less severe reduction in ISO and HIS response occurred in normal subjects during an experimental rhinovirus 16 infection (J Allergy Clin Immunol, in press).

Impairment in PMN response to the agonists, ISO, HIS, and PGE, (10-8 to 10-4M) develops during in vitro incubation with several respiratory viruses: rhinovirus 16, influenza A (England/42/72[H3N2]), and two live attenuated influenza vaccines (bivalent: A [H3N2] and B; WRL-105 type A). Bivalent vaccine affects PMNs from normal (n=15) and asthmatic (n=29) donors similarly. Asthmatics with a history of virus-provoked episodes of wheezing with colds did not differ from other asthmatics. Vaccine did not impair agonist response on PMNs collected from patients receiving systemic corticosteroids and in vitro cortisol (50×10-4M) restored the vaccine-induced impairment in response to agonists.

Preliminary observations show the change in agonist response is proportional to the concentrations of the virus preparation, is retained following virus inactivation (heat, ultraviolet light), and is restored by washing after virus incubation.

DISCUSSION

Dr. Stechschulte: Do you have any evidence that phosphodiesterase activity or that cyclic nucleotide levels are altered by the 30 minute incubation?

Dr. Busse: The baseline levels of cAMP are not changed. We do not have measurements of phosphodiesterase activity.

Dr. Oren: Are these effects you see with viral incubation due to infection or to some other mechanism, perhaps immunologic?

Dr. Busse: In studies in which the viruses have been killed we see the same results as with the live virus. Whether it is some viral product or an effect of the virus particle itself, we do not know.

Dr. Chester: Have you had an opportunity to do in vitro studies on leukocytes from non-asthmatic normal subjects during and after viral infections?

Dr. Busse: Yes, and they were similarly affected.

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