It has been postulated that the patient with atopic dermatitis has defective beta adrenergic receptor function. However, a more generalized defect is suggested by the observation that cyclic AMP generation is diminished in these patients following stimulation with both isoproterenol and PGE\textsubscript{2}. To determine the nature of this abnormality, we measured beta adrenergic receptor binding directly on polymorphonuclear leukocyte membranes using the radiolabeled beta adrenergic antagonist (−) [\textsuperscript{3}H]dihydroalprenolol (DHA). DHA binding was studied in 6 mild and 9 moderate-to-severe atopic dermatitis patients, and 8 normal controls using a subsaturating concentration of DHA (0.5 nm) to estimate receptor affinity and a saturating concentration of DHA (30 nm) to determine the total number of receptors per cell.

No significant differences (p > .05) were found in the total number of receptors per PMN between the control population (805 ± 95) and the mild atopic dermatitis patients (745 ± 91) or the moderate to severe group (621 ± 79). In addition, no significant differences in receptor affinity were found among any of the 3 study groups. These findings suggest that beta receptor binding in atopic dermatitis is normal. Reduced cyclic AMP generation in atopic dermatitis PMN leukocytes would appear to be due to a defect distal to the beta adrenergic receptor itself.

Szentivanyi [1] has postulated that atopic disease is characterized by a blockade of the beta adrenergic receptor resulting in an imbalance with increased alpha adrenergic and cholinergic receptor function. Clinical and laboratory evidence suggest that an imbalance of the autonomic nervous system exists in atopic dermatitis and that this phenomenon may be important in the pathogenesis of the disease [2]. Recently, impaired beta adrenergic function in peripheral blood leukocytes of patients with active atopic dermatitis has been reported [3,4]. Busse and Lee [3] reported decreased cyclic adenosine 3,5 monophosphate (cyclic AMP) responses to isoproterenol in granulocytes and lymphocytes from eczema patients, while Parker, Kennedy, and Ehein [4] showed a decreased response to both isoproterenol and prostaglandin (PG) E\textsubscript{1}, in leukocytes and purified lymphocytes in patient with severe, but not mild atopic dermatitis. These studies suggest that if defective generation of cyclic AMP truly exists in atopic disease, the defect may be distal to the beta adrenergic receptor since a decreased response was seen following stimulation with PGE\textsubscript{2}, as well as isoproterenol.

In order to more clearly identify the abnormality in the pathway to cyclic AMP generation, one must assess the first step in the sequence, that of hormone binding to the beta adrenergic receptor. We have recently developed a method for the direct measurement of the number and affinity of beta adrenergic receptors on polymorphonuclear (PMN) membranes using the ligated beta adrenergic antagonist (−) [\textsuperscript{3}H]dihydroalprenolol (DHA) [5]. PMN may be more suitable than lymphocytes for clinical studies of beta adrenergic receptors, because PMN appear to be a more homogeneous population than are lymphocytes [6-8]. In this study we report our findings of beta adrenergic receptor binding in mild and moderate to severe atopic dermatitis patients compared to a healthy control group.

MATERIALS AND METHODS

Subjects

A total of 15 patients with atopic dermatitis and 8 normal controls were studied. The diagnosis of atopic dermatitis was based on well-defined clinical and historical features as previously described [2]. Extent (E) and severity (S) of disease were graded on a scale of 1−5 and the product of the 2 grades (E × S) was the overall indicator of clinical involvement [9]. Thus, a score of 25 would indicate maximally severe generalized erythroderma. Patients with E × S scores of 10 or under were considered to have mild atopic dermatitis while those with scores greater than 10 were defined as having moderate to severe atopic dermatitis. Subjects were divided into three categories as follows.

Group I—control population: There were 8 control subjects who had no evidence of atopic dermatitis or other atopic diseases. These subjects included 5 males and 3 females with a mean age of 36.9 yr for the group.

Group II—mild atopic dermatitis (clinical score ≤10): This group consisted of 5 patients, 2 males and 4 females with a mean age of 31.8 yr.

Group III—moderate-to-severe atopic dermatitis (clinical score >10): There were 9 patients in this group, 3 males and 6 females, with a mean age of 34.1 yr.

Selective Criteria

The subjects in all 3 groups were carefully screened to exclude individuals with active viral infection, those receiving adrenocorticosteroids, cardiac glycosides, antihypertensive or other sympathomimetic-containing medication.

Preparation of Polymorphonuclear (PMN) Particulates

Sixty ml of heparinized blood was obtained from each subject in the study. Blood was centrifuged on Ficoll-Hypaque density gradient according to the method of Boyum [10]. The pellets containing erythrocytes and granulocytes were diluted 1:2 with phosphate-buffered saline (PBS) and then mixed with an equal volume of 3% (wt/vol) high molecular weight Dextran (T250 Pharmacia Fine Chemicals, Piscataway, New Jersey) in PBS. The erythrocytes were allowed to settle for 25 min at room temperature, and the Dextran separation procedure was repeated since this second separation increases the PMN yield. The supernatant fraction was then exposed to 0.2% NaCl for 20 seconds in order to lyse contaminating erythrocytes; 1.6% NaCl was used to restore isotonicity, and the cells were centrifuged at 350 × g for 10 min. The cellular pellet was resuspended in a few ml of PBS and the number of cells was counted. PMN were 90 ± 3.5% (mean ± SD) with the remainder of the cells less than 5% lymphocytes, less than 5% eosinophils, less than 1% basophils, and less than 1% monocytes. Less than 1 platelet was found for every nucleated cell counted. The cellular suspension was made hypotonic with 0.2% NaCl for 30 to 40 min at 4°C, and then centrifuged at 450 × g for 10 min. The pellet was resuspended in incubation buffer (50 mM Tris HCL (pH 8.1 at 4°C), 10 mM MgCl\textsubscript{2}) at a density equivalent to 10\textsuperscript{6} cells/ml. The suspension was subjected to freeze thawing and then

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Reprint requests to: S. P. Galant, M.D., Department of Pediatrics, Room 2E 502 Medical Center, University of Utah College of Medicine, Salt Lake City, Utah 84132.

Abbreviations:
DHA: (−) [\textsuperscript{3}H]dihydroalprenolol
PBS: phosphate buffered saline
PMN: polymorphonuclear leukocytes

The Department of Pediatrics, University of Utah, Salt Lake City, and the Department of Dermatology, University of Oregon, Portland
homogenized with 8 to 10 strokes in a Ten-Broek homogenizer prior to use in the binding assay. All particulates were used in the binding assay immediately after thawing and homogenization.

**Beta-Adrenergic Receptor Binding Assay**

In a previous study [5], we found that the dissociation constant (K_D) for DHA binding to PMN particulates was 1 to 5 nM and, thus, saturation of the binding sites occurred at 10 to 30 nM DHA. In the present study we used two DHA concentrations, 0.5 nM and 30 nM for all subjects. Because of the yield of PMN particulate protein it was impossible to study DHA binding at more than 2 DHA concentrations. We chose 0.5 nM as a subsaturating concentration and 30 nM as a concentration that would reflect the total number of DHA binding sites. DHA (New England Nuclear) specific activity was 49 Ci/mmol.

Binding assays contained in the total volume of incubation buffer of 0.15 ml of ice-cold incubation buffer and the radioactivity of the dried supernatant after homogenization was 40 μg ml (± 1 mg protein). Each assay contained corresponding to 440 μg ml protein, 1 mM ascorbic acid, 0.3 mM catechol, and 0.1 mM phenolamine. Ascorbic acid is included to block oxidation of catecholamines, and catechol and phenolamine to inhibit nonspecific binding [11,12]. These agents did not affect specific binding.

Samples were incubated at 37°C for 15 min. Incubations were terminated by rapidly diluting samples with 2 ml of ice-cold incubation buffer and then immediately filtering the mixture through a glass filter (Gelman A/E). The filters were prewashed in incubation buffer containing 0.1 ml 2% propanol to block nonspecific binding of radioactivity to the filter. The filters were rapidly (10 seconds) washed with 15 ml of ice-cold incubation buffer and the radioactivity of the dried filters was determined in a liquid scintillation system (β efficiency = 45%). Specific binding represents the total amount of radioactive DHA bound minus the amount bound in the presence of 1 μM (±)-propranolol and was greater than 80% at 0.5 nM and about 50% at 30 nM of the total amount bound. All data shown in this paper are specific binding.

Specific binding was linear with protein at the concentration used in these experiments. All samples were run as duplicates and these differed from each other by less than 15%. Protein was measured by the method of Lowry, Rosebrough, and Farr [13] using bovine serum albumin standards.

**Analysis of Binding Data**

The quantity of DHA binding in fmol/mg protein was determined. Since 1 mg of our particulate preparation is derived from 1.8 × 10^7 PMN, the number of receptors per cell was calculated by the formula:

\[
\text{Number of receptors} = \frac{\text{moles of DHA bound}}{\text{cell}} \times \frac{6.02 \times 10^{23} \text{molecules}}{\text{mole}}
\]

Each group was compared with the others using a nonpaired Student’s test analysis for significant differences in binding.

**RESULTS**

Although the ratio of males to females was slightly different in Group I (5:3) compared to Groups II and III (2:4 and 3:6 respectively) we have previously shown no difference in DHA binding between males and females [14]. In addition, the groups appear reasonably well age matched with mean ages of 36.9 years for Group I compared to 31.8 years in Group II and 34.1 years in Group III. DHA binding in the normal subjects (Group I) was compared with the mild atopic dermatitis patients (Group II) and those with moderate-to-severe disease (Group III) both at the low DHA concentration (0.5 nM) and at the saturation DHA concentration (30 nM) in the Table. Statistical analysis revealed no significant difference (p >.05) between the control population and either atopic dermatitis group. This was true at 0.5 nM when one compares Group I at 9.7 ± 1.2 fmol/mg protein (mean ± SEM) with Group II at 9.5 ± 1.7 or Group III with 9.2 ± 0.9, and at the 30 nM DHA concentration where the control group bound 25.4 ± 3.0 fmol/mg protein compared to 23.5 ± 3.0 and 19.6 ± 2.5 for the mild and moderate-to-severe atopic dermatitis groups respectively. The total number of receptors was calculated for each group. Group I PMN had 805 ± 95 beta adrenergic receptors per cell compared to 745 ± 91 and 621 ± 79 PMN receptors for Group II and III. Again, no statistical difference (p >.05) was found between any of the group comparisons. Finally, if one uses the percentage of the total binding at 0.5 nM DHA as an indication of receptor affinity, no significant differences are found between groups.

**DISCUSSION**

In this report we have found that the PMN beta adrenergic receptor in atopic dermatitis cannot be distinguished statistically from a normal control population regardless of the disease severity. However, since DHA dose response curves were not performed on atopic dermatitis patients because of the blood requirement we have not ruled out the possibility of abnormal DHA binding kinetics in this population. Although this question cannot be presently resolved from this study, we feel our data certainly suggest that the total number of receptors and the estimate of receptor affinity in atopic dermatitis are both normal. Patient 3 in Group III has low binding, but he too falls within 2 standard deviations of the mean DHA binding found in our laboratory [14]. Although the number of subjects is small, these data are very similar to those we have recently reported in a larger number of patients with bronchial asthma [14,15]. Asthmatics have normal binding regardless of the severity of their illness unless they are receiving adrenergic drug therapy. Patients with severe atopic dermatitis appear to have normal binding too, although Parker, Kennedy, and Eisen [4] clearly showed significantly decreased cyclic AMP responses to both isoproterenol and PGE1 in leukocytes from patients with severe but not mild disease. Although these data appear to be in conflict at first, they may be in fact consistent with a defect in the coupling of the membrane-hormone receptor with the enzyme adenylate cyclase rather than an abnormality of the receptor itself. This would account for the decreased cyclic

<table>
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<th>Group I—Normals</th>
<th>Group II—Mild atopic dermatitis</th>
<th>Group III—Moderate to severe atopic dermatitis</th>
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<tr>
<td>DHA binding (fmol/mg protein)</td>
<td>DHA binding (fmol/mg protein)</td>
<td>DHA binding (fmol/mg protein)</td>
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<tr>
<td>Subject</td>
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<td>38 M</td>
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<td>P* vs. Grp II</td>
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* Clinical atopic dermatitis score determined by extent and severity of lesions (see Material and Methods).

* P values determined by nonpaired student t-test.
AMP response not only to isoproterenol, but PGE$_1$ as well. In addition, in bronchial asthma an analogous situation may exist explaining the diminished granulocytes cyclic AMP responsiveness to both histamine [16] and isoproterenol [17].

Atopic dermatitis would appear to provide an excellent model for study of the proposed abnormality of the beta adrenergic system since, unlike asthma, systemic drugs are seldom necessary and abnormalities in leukocyte function including the granulocyte have been identified [18]. Measurement of beta receptor binding and adenylate cyclase activity on the same membrane preparation in a larger number of patients should help elucidate the nature of the diminished cyclic AMP responsiveness. Hopefully these studies will improve our understanding of the pathogenesis of atopic dermatitis as well as other atopic diseases.

REFERENCES
14. Galant SP, Durisetti L, Underwood S, Insel PA: Beta adrenergic receptors of polymorphonuclear particulates in bronchial asthma, submitted for publication

Announcement

Practical Skin Pathology

A course in skin pathology, sponsored by the departments of dermatology and pathology, New York Medical College-Metropolitan Hospital Center; the American Society of Clinical Pathology; and the American Society of Dermatopathology, will be given on August 26 through 31, 1979 at Grossinger’s, Grossinger, New York. The course will be a comprehensive, practical review, and update of dermatopathology. It will consist of formal lectures, question and answer periods, supervised microscope sessions, opportunity for optional independent study of microscopic slides, and a post test period with in-depth panel discussions. The course is accredited for 39 hours in category 1 toward the AAD Continuing Education Award. For further information, please write to: Martin H. Brownstein, M.D., Skin Pathology Course, Two Jordan Drive, Great Neck, New York, 11021.