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Stanley P. Galant, … , Sandra Allred, Paul A. Insel

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**Research Article**

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In further studies we examined the interaction of the agonist (–)-isoproterenol with beta adrenergic receptors in 8 normal subjects and 10 asthmatics not receiving adrenergic medication. We tested the ability of isoproterenol to compete for DHA binding sites and to stimulate adenylate cyclase in sonicates prepared from PMN and examined under identical conditions. The dissociation constants for the competition of isoproterenol […]



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STANLEY P. GALANT, LAKSHMI DURISETI, SHARON UNDERWOOD, SANDRA ALLRED, and PAUL A. INSEL, *Department of Pediatrics, University of California, Irvine, Irvine, California 92717; Department of Pediatrics, University of Utah, Salt Lake City, Utah 84132; Division of Pharmacology, Department of Medicine, University of California, San Diego, San Diego, California 92093*

ABSTRACT We have tested the beta adrenergic receptor theory of bronchial asthma by determining the number and affinity of binding sites of the beta adrenergic radioligand [$^3$H]dihydroalprenolol (DHA) and the activity of adenylate cyclase in broken cell preparations of polymorphonuclear leukocytes (PMN). We studied 31 control subjects (group 1), 30 asthmatics receiving no systemic adrenergic medication (group 2), and 17 asthmatics receiving adrenergic agonists systemically (group 3). Control subjects and asthmatics taking no adrenergic drugs bound similar amounts of DHA at 0.5 nM and 30 nM DHA and had about 900 binding sites per PMN. In contrast, asthmatics receiving adrenergic agonists had a >70% decrease in their number of DHA binding sites per PMN (254±57). In a subset of our three groups of subjects (eight from group 1, six from group 2, and five from group 3) we measured DHA binding at several DHA concentrations and found similar values (0.4–0.7 nM) for the dissociation constant of DHA among these subjects.

In further studies we examined the interaction of the agonist (−)-isoproterenol with beta adrenergic receptors in 8 normal subjects and 10 asthmatics not receiving adrenergic medication. We tested the ability of isoproterenol to compete for DHA binding sites and to stimulate adenylate cyclase in sonicates prepared from PMN and examined under identical conditions. The dissociation constants for the competition of isoproterenol for DHA binding sites in normal and asthmatic subjects were virtually identical (~1.0 $\mu$M). In addition, the (activation constant) values for stimulation of adenylate cyclase were similar (0.16–0.19 $\mu$M) in the two groups of subjects.

Thus, these data suggest that asthma per se is not associated with alteration in either the number or affinity of beta adrenergic receptors in PMN. Our findings indicate that previous reports of abnormal beta adrenergic receptor function in asthmatic patients may in part be explained by prior treatment of such patients with adrenergic agonists. Because the asthmatics who received adrenergic agonists in our study tended to be more ill and to receive additional medication compared to subjects in group 2, we cannot rule out unequivocally that severe asthma may be associated with decreased binding to beta adrenergic receptors. Nevertheless, we conclude that beta adrenergic receptors on PMN from asthmatics are relatively normal unless such patients are treated with adrenergic agonists.

## INTRODUCTION

In 1968, Szentivanyi (1) postulated that the underlying defect in atopic diseases, particularly bronchial asthma, is an imbalance of the autonomic nervous system. He suggested that beta adrenergic receptor function is decreased in asthmatic patients and that this results in increased alpha adrenergic and cholinergic responses to a variety of stimuli. Certain evidence has been reported that appears to confirm the possibility of beta adrenergic blockade in pulmonary (2, 3) and extrapulmonary tissues in asthmatics (4). A consequence of this blockade would be decreased cellular cyclic AMP, which in the lung would promote bronchospasm and mediator release (5, 6). Because it is difficult to study samples of lung in human subjects, several investigators have used either peripheral blood leukocytes or lymphocytes to show that asthmatics have decreased stimulation of cyclic AMP by beta adrenergic agonists (7, 8). Two problems must be considered in

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assessing these studies: (*a*) beta adrenergic responses and receptors in T and B lymphocytes may be quite different (9, 10), and thus changes in adrenergic response in asthmatics might reflect altered distribution of cells in lymphocyte subpopulations; and (*b*) prior administration of beta adrenergic agonists reportedly diminishes subsequent beta adrenergic stimulation of leukocyte cyclic AMP (11–13). Thus, changes in responsiveness of asthmatics might arise from therapy as well as from the disease itself. Some authors (7) have attempted to consider these problems in studies with lymphocytes.

Neutrophilic polymorphonuclear cells (PMN)[1] 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. In addition, PMN have well-defined beta adrenergic responses that have been reported to be decreased in asthmatics (14–16). Recently we have used [$^3$H]dihydroalprenolol (DHA), a radiolabeled beta adrenergic antagonist to characterize beta adrenergic binding sites (i.e., receptors) on PMN particulates prepared from normal subjects (17). In this study we have compared DHA binding of control subjects with binding of asthmatics who either were or were not receiving adrenergic agents. In addition, we have compared the ability of the agonist isoproterenol to compete for DHA binding sites and to stimulate adenylate cyclase in broken PMN from controls and asthmatics. We find that asthmatics have only minimal alterations in PMN beta adrenergic receptors unless the patients are receiving therapy with beta adrenergic agonists.

## METHODS

### *Subjects*

SUBJECTS WERE DIVIDED INTO THREE CATEGORIES

*Group 1: control population.* These were 31 healthy adults without evidence of atopic disease or asthma. The age ranged from 19 to 65 yr (mean age, 34) with 13 males and 18 females.

*Group 2: asthmatics receiving no beta adrenergic medication.* There were 30 asthmatics in this category who had received no beta adrenergic drugs systemically for at least 2 wk before the study (Table I). Three patients (Nos. 20, 28, and 29) used a preparation of inhaled metaproterenol infrequently and had not used it for 48 h before testing. Patients in this group were allowed theophylline, cromolyn sodium, or corticosteroids as necessary. This group was comprised of 15 males and 15 females with an age range from 15 to 64 (mean age, 36). The duration of asthma ranged from 1 to 40 yr (mean, 16 yr).

*Group 3: asthmatics receiving beta adrenergic medication.* This group consisted of 17 patients who were receiving beta adrenergic medication at therapeutic dosage in addition to the medication mentioned in group 2 (Table II). There were nine males and eight females with an age range of 18 to 60 (mean age, 35 yr). The duration of asthma ranged from 1 to 58 yr (mean, 17 yr).

### *Selection of criteria*

The subjects in all three groups were carefully screened to exclude individuals with active viral infections, those receiving birth control medication, cardiac glycosides, antihypertensive agents, or other sympathomimetic containing medication. All asthmatics in this study had previously demonstrated reversible airway disease by pulmonary function testing.

### *Grading of asthma severity and activity*

Severity of disease was graded on the basis of the overall course of asthma with the following criteria: (*a*) steroid usage, (*b*) requirement for bronchodilation therapy to control asthma, (*c*) frequency and persistence of asthma exacerbations, and (*d*) hospitalization. From these criteria asthmatics were identified as mild, moderate, or severe. Mild asthmatics could be controlled with intermittent bronchodilator therapy. Patients in the moderate category required daily bronchodilator therapy and short courses of steroids for acute exacerbations. The severe asthmatics required frequent treatment (more than one course per month) with steroids, daily bronchodilators, and had persistent wheezing with frequent exacerbations of asthma often requiring hospitalization. The activity of disease was scored as 0 to 3, where 0 meant that no wheezing had occurred within 3 mo, 1 indicated wheezing between 14 d and 3 mo, 2 signified wheezing within 2 wk, and 3 indicated acute asthma at the time of the study.

### *Preparation of PMN particulates*

60 ml of heparinized blood was obtained from each subject in the study. Patients on adrenergic therapy were sampled generally 2–4 h after taking their medication. Blood was centrifuged on a Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N. J.) density gradient according to the method of Böyum (18). Particulates from PMN (90±3.5% SD of final cells obtained) were prepared as described (17) or by sonicating (Heat Systems-Ultrasonics, Inc., Plainview, N. Y.) cells gently at a setting of two for 5 s. Freshly prepared particulates or sonicates were used immediately in the binding studies and sonicates were used immediately in adenylate cyclase assays.

### *Beta adrenergic receptor assay*

DHA Binding assays contained in a total volume of 0.15 ml: DHA, PMN membranes (~0.440 mg protein in which 1 mg protein corresponds to $1.7 \times 10^7$ cells), 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM ascorbic acid, 0.3 mM catechol, and 0.1 mM phentolamine. Ascorbic acid was included to block oxidation of catecholamines, and catechol and phentolamine were added to inhibit nonspecific binding (17). 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 fiber filter (Gelman A/E, Gelman Instruments Co., Ann Arbor, Mich.). The filters were presoaked in incubation buffer containing 0.1 mM (±)-propranolol to block nonspecific binding of radioactivity to the filters. The filters were rapidly (10 s) washed with 15 ml of ice-cold incubation buffer and the radioactivity

[1] *Abbreviations used in this paper*: DHA, [$^3$H]dihydroalprenolol; PMN, polymorphonuclear cells.

TABLE I

*Asthmatics not Receiving Beta Adrenergic Drugs (Group 2)*

| Subject | Age | Sex | Duration | Severity | Activity | Medications |
|---|---|---|---|---|---|---|
| | | | *yr* | | | |
| 1 | 50 | M | 25 | Severe | 3 | Theophylline, beclomethasone dipropionate aerosol |
| 2 | 34 | M | 30 | Mild | 1 | None |
| 3 | 46 | M | 40 | Mild | 0 | None |
| 4 | 30 | F | 17 | Moderate | 2 | Theophylline |
| 5 | 23 | F | 8 | Moderate | 2 | Theophylline |
| 6 | 28 | M | 2 | Moderate | 2 | Theophylline, cromolyn sodium |
| 7 | 45 | F | 40 | Mild | 2 | None |
| 8 | 40 | F | 20 | Moderate | 1 | Theophylline |
| 9 | 31 | F | 30 | Moderate | 2 | Theophylline |
| 10 | 31 | M | 30 | Moderate | 2 | Theophylline |
| 11 | 33 | M | 10 | Mild | 1 | None |
| 12 | 36 | F | 30 | Moderate | 2 | Theophylline |
| 13 | 15 | F | 14 | Moderate | 2 | Theophylline |
| 14 | 52 | F | 4 | Severe | 2 | Theophylline |
| 15 | 28 | F | 25 | Mild | 2 | None |
| 16 | 50 | F | 1 | Moderate | 2 | Theophylline |
| 17 | 25 | M | 6 | Mild | 0 | None |
| 18 | 28 | M | 20 | Mild | 1 | None |
| 19 | 29 | M | 24 | Mild | 3 | None |
| 20 | 31 | F | 1 | Moderate | 3 | Theophylline, metaproterenol inhaler, beclomethasone dipropionate inhaler |
| 21 | 41 | M | 18 | Mild | 3 | None |
| 22 | 28 | F | 9 | Mild | 3 | None |
| 23 | 32 | M | 20 | Moderate | 1 | Theophylline |
| 24 | 39 | M | 1 | Mild | 0 | None |
| 25 | 50 | M | 6 | Moderate | 1 | Theophylline beclomethasone dipropionate inhaler |
| 26 | 38 | F | 20 | Moderate | 1 | Theophylline, beclomethasone dipropionate |
| 27 | 41 | F | 10 | Severe | 2 | Theophylline, prednisone |
| 28 | 29 | F | 20 | Moderate | 2 | Theophylline, metaproterenol inhaler |
| 29 | 44 | M | 4 | Severe | 2 | Theophylline, prednisone, metaproterenol, beclomethasone dipropionate |
| 30 | 64 | M | 2 | Severe | 3 | Theophylline, beclomethasone dipropionate |

of the dried filters was determined in a liquid scintillation system ($^3H$ efficiency = 45%). Specific binding represents the total amount of radioactive DHA bound minus the amount of DHA bound in the presence of 1 $\mu$M (±)-propranolol; specific binding was 50–80% of the total amount bound at all DHA concentrations. All data shown in this paper are specific binding. Specific binding was linear with protein over the concentration range used in these experiments. All samples were run as duplicates and these differed from each other by <15%. Protein was measured by the method of Lowry et al. (19) with bovine serum albumin standards.

## *Adenylate cyclase assays*

In this study, adenylate cyclase assays were performed concurrently with DHA binding studies under identical conditions. Samples were incubated in a total volume of 0.17 ml containing Tris-HCl, pH 7.5, 50 mM; $MgCl_2$, 5 mM; dithiothreitol, 1 mM; [$\alpha$-$^{32}$P]ATP, 1 mM and 1–2 × $10^6$ cpm; cyclic AMP, 0.5 mM; 3-isobutyl-1-methylxanthine, 0.2 mM; phosphoenolpyruvate, 16 mM; pyruvate kinase, 50 $\mu$g/ml; ascorbic acid, 1 mM; catechol, 0.3 mM; phentolamine, 0.1 mM; DHA, 10 nM; PMN sonicate protein, 0.30–0.45 mg, and various additions as indicated. Routinely, samples were incubated with all reactants except [$^{32}$P]ATP (but including 1 mM nonradioactive ATP) for 15 min at 37°C, at which time equilibrium of DHA binding was achieved. [$^{32}$P]ATP was then added to the samples in some experiments and the incubation was continued for an additional 15 min. Samples for DHA binding were then filtered and washed as described above, and in those experiments in which [$^{32}$P]ATP had been added, the total filter-bound radioactivity was corrected for counts of $^{32}$P retained on the filters (generally <100 cpm). For samples in which adenylate cyclase activity was measured, 0.15 ml of 0.33 N HCl was added and cyclic AMP was then isolated by sequen-

TABLE II

*Asthmatic Receiving Beta Adrenergic Drugs (Group 3)*

| Subject | Age | Sex | Duration | Severity | Activity | Medications |
|---|---|---|---|---|---|---|
| | | | *yr* | | | |
| 1 | 47 | M | 1 | Severe | 2 | Terbutaline 5 mg/q6h, theophylline |
| 2 | 37 | M | 35 | Severe | 3 | Terbutaline 2.5 mg q6h, theophylline, prednisone |
| 3 | 32 | M | 2 | Severe | 2 | Terbutaline 5 mg q6h, theophylline, cromolyn sodium, prednisone qod |
| 4 | 60 | F | 58 | Severe | 3 | Terbutaline 5 mg tid, metaproterenol inhaler (2) tid, theophylline, methylprednisolone |
| 5 | 31 | F | 30 | Severe | 2 | Terbutaline 5 mg q8h, theophylline, prednisone qod, beclomethasone dipropionate aerosol |
| 6 | 37 | M | 20 | Mild | 2 | Terbutaline 2.5 mg bid |
| 7 | 47 | M | 18 | Severe | 2 | Terbutaline 2.5 mg qid |
| 8 | 47 | M | 10 | Severe | 2 | Ephedrine 25 mg q6h, theophylline, cortisone daily, beclomethasone dipropionate |
| 9 | 20 | F | 3 | Severe | 2 | Ephedrine 25 mg qid, metaproterenol inhaler (1–2) qid |
| 10 | 24 | M | 20 | Severe | 2 | Terbutaline 2.5 mg q6h, theophylline |
| 11 | 30 | F | 22 | Severe | 2 | Metaproterenol (20 mg) 1 tid isoetharine inhaler bid, theophylline, beclomethasone dipropionate aerosol |
| 12 | 36 | F | 23 | Severe | 2 | Terbutaline 5 mg tid, theophylline, beclomethasone dipropionate aerosol |
| 13 | 35 | M | 7 | Severe | 3 | Terbutaline 5 mg tid, fenoterol inhaler tid, metaproterenol inhaler tid, theophylline, beclomethasone dipropionate inhaler, prednisone |
| 14 | 50 | M | 8 | Moderate | 2 | Terbutaline 5 mg tid, prednisone |
| 15 | 18 | F | 10 | Moderate | 3 | Terbutaline 5 mg tid, beclomethasone dipropionate inhaler, metaproterenol |
| 16 | 18 | F | 5 | Moderate | 2 | Terbutaline 5 mg tid, beclomethasone dipropionate inhaler, theophylline |
| 17 | 25 | F | 23 | Severe | 3 | Terbutaline 5 mg tid, prednisone, theophylline |

tial chromatography on Dowex AG 50 WX4 and neutral alumina, as described by Salomon et al. (20). Recovery of cyclic [$^{32}$P]AMP (determined by addition of cyclic [$^{3}$H]AMP) was generally 70–90%. Radioactivity was measured in eluates from the alumina column counted in Aquasol-2 solution (New England Nuclear, Boston, Mass.) using a Beckman LS 8000 scintillation counter (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Adenylate cyclase activity was linear for 30 min at 37°C at the concentrations of protein used in these experiments. All experiments were performed in duplicate, and the variability between samples was usually between 10 and 15% or less. Under the conditions used, the maximal level of isoproterenol-stimulated adenylate cyclase activity was generally 100–150 pmol cyclic AMP generated/min per mg protein and the specific activity of DHA bound was 10–15 fmol/mg protein.

Competition of isoproterenol for DHA binding sites was expressed as a percentage of that competed by 1 $\mu$M (±)-propranolol (i.e., 100% specific DHA binding). The dissociation constant for isoproterenol ($K_d$ isoproterenol) was calculated as described by Cheng and Prusoff (21) with the equation:

$$K_d \text{ isoproterenol} = I_{50}\left(\frac{K_d \text{ DHA}}{S + K_d \text{ DHA}}\right),$$

where $I_{50}$ means the concentration of isoproterenol competing for 50% of the specific DHA binding sites, $K_d$ DHA was assumed to be 0.37 and 0.39 nM in asthmatics and controls, respectively (see below), and S was 10 nM, the DHA concentration used in these experiments.

The potency of (−)-isoproterenol in stimulating adenylate cyclase activity was determined graphically and expressed as the $K_{act}$, where the observed concentration producing a 50% maximal stimulation ($EC_{50}$) was corrected for the DHA included in the incubation by the equation shown above.

## *Materials*

Most reagents were obtained from sources listed previously (17). Dithiothreitol, phosphoenolpyruvate, cyclic AMP, ATP, and neutral alumina were purchased from Sigma Chemical Co. (St. Louis, Mo.) Dowex AG 50Wx4, other reagents were

purchased as follows: Bio-Rad Laboratories (Richmond, Calif.); pyruvate kinase, Boehringer Mannheim Biochemicals (Indianapolis, Ind.); [$^{32}$P]ATP (10 Ci/mmol) and [$^{3}$H]cyclic AMP (37.7 Ci/mmol), New England Nuclear, and 3-isobutyl-1-methylxanthine, Aldrich Chemical Co., Inc. (Milwaukee, Wis.).

## RESULTS

*Clinical data.* The clinical data of asthmatic patients on nonadrenergic drugs (group 2) is shown in Table I, whereas data for those taking adrenergic and nonadrenergic drugs (group 3) is in Table II. The patients appeared to be reasonably well-matched for age, sex, and duration of illness, as noted above. Subjects in group 3 had more severe disease (13 of 17 subjects vs. 5 of 22 in group 2) and greater activity of their asthma than those in group 2 (disease activity scores of 1.77±0.87, mean±SD, for group 2 and 2.29±0.47 for group 3, one-tailed $P < 0.05$). 12 patients in group 3, and 7 in group 2 received corticosteroids, and 12 of 17 patients in group 3 and 19 of 30 patients in group 2 received theophylline derivatives. The most commonly used adrenergic agent by the patients in group 3 was terbutaline, which 14 of the 17 patients ingested at doses ranging from 5 to 20 mg/d. Other adrenergic drugs used by patients in group 3 included ephedrine, metaproterenol, isoproterenol, fenoterol, and isoetharine.

*DHA binding studies.* To determine properties of beta adrenergic receptors in the three groups of subjects, we conducted studies to examine receptor binding (affinity and number of receptors per PMN) as well as receptor function (activation of adenylate cyclase). In studies of receptor affinity we performed DHA binding experiments in eight control subjects, six asthmatics receiving no adrenergic medication (subjects 17–22, Table I), and 5 asthmatics receiving adrenergic agonists (subjects 13–17, Table II). Mean data for these three groups are shown in Fig. 1. Specific binding was saturated and could be resolved into one class of binding sites ($r$ by least squares fit = 0.94, 0.99, and 0.96 for groups 1, 2, and 3, respectively) on Scatchard analysis (Fig. 1, inset). The mean data shown in Fig. 1 yield a dissociation constant for DHA of 0.39, 0.30, and 0.49 nM for groups 1, 2, and 3, respectively. When individual studies on the 19 subjects were analyzed by Scatchard plots (all $r$ values for regression lines were ≥0.80) and the individual dissociation constants pooled for the three groups, the population values were 0.41±0.05, 0.38±0.06, and 0.72±0.22 nM (mean±SEM). The values for the dissociation constant for the subjects in group 3 were not statistically different when compared to those for subjects in group 1 or 2 ($P > 0.05$). These values for the dissociation constant for DHA are somewhat lower than we reported previously (1–5 nM [17]) and may reflect the fact that the data in Fig. 1 was obtained using sonicates rather than with membrane particulates, which were used in our earlier study and in the studies shown in Fig. 2.

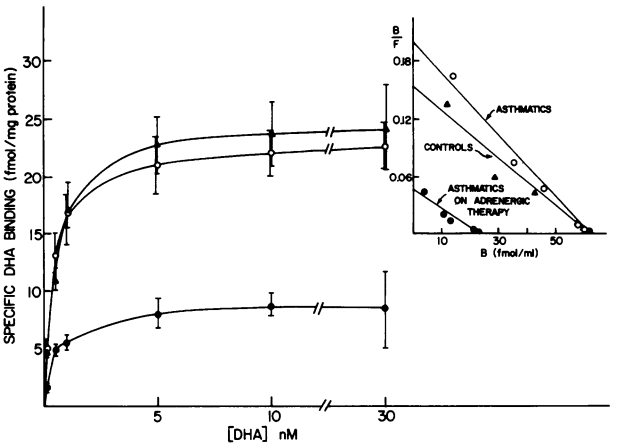


FIGURE 1 Saturability of DHA binding to PMN particulates. Specific DHA bound (femtomoles per milligram protein) to PMN particulates from eight control subjects (▲), six asthmatics not receiving systemic adrenergic agonists (○), and five asthmatics receiving adrenergic agonists (●) is plotted on the ordinate, and the total DHA concentration in each sample is on the abscissa. The data shown are the mean±SEM. The inset shows a Scatchard plot of DHA bound (femtomoles per milliliter) using the mean data from the figure for each group of subjects. The lines in the inset are the regression lines determined by least squares fit for each group (values stated in text).

Data for maximal number of DHA binding sites in these subjects were obtained in two ways: (*a*) Scatchard analysis of data for the subjects depicted in Fig. 1 and (*b*) DHA binding determined in a larger group of 26 controls, 22 subjects in group 2 (subjects 1–22), and 17 subjects in group 3 at a single concentration of DHA (30 nM). This concentration appears to be close to saturating for these three groups of subjects (Fig. 1 and [17]).

Data derived from the Scatchard analyses yield values of 24.2±3.2, 23.1±2.0, and 10.1±1.2 fmol/mg

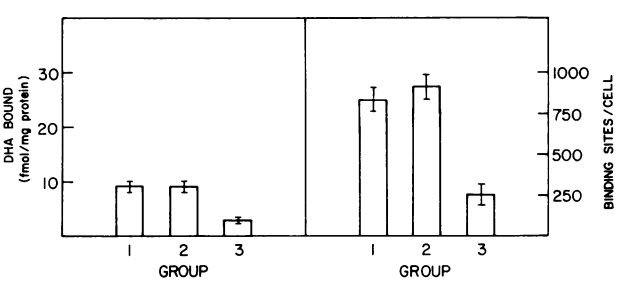


FIGURE 2 DHA binding to PMN particulates at 0.5 nM (left panel) and 30 nM DHA (right panel). DHA binding to PMN particulates is plotted as either femtomoles per milligram protein or as binding sites per cell for 26 control subjects, 22 asthmatics receiving no systemic adrenergic therapy, and 17 asthmatics receiving adrenergic agonists. The data shown are mean±SEM for each group.

PMN particulate protein (mean±SEM) in groups 1, 2, and 3, respectively. The number of binding sites in group 3 was significantly lower ($P < 0.005$) than that in either group 1 or 2.

The values for the DHA binding studies in each of the three groups of subjects performed at 30 nM DHA (Fig. 2, right panel) were 25.1±1.8, 27.6±1.7, and 7.6±1.7 (mean±SEM) for controls, asthmatics taking no adrenergic agonists, and asthmatics receiving adrenergic agonists. These values correspond to 838±60, 922 ±57, and 254±57 binding sites per PMN in the three groups. Values for control subjects and asthmatics receiving no adrenergic medication were similar ($P > 0.1$), whereas asthmatics receiving asthmatic agonists had significantly less binding at 30 nM DHA ($P < 0.001$ compared to both of the other groups). The amount of DHA bound at 30 nM DHA was not significantly correlated with duration of asthma in patients in either group 2 or 3 ($P > 0.05$). Subjects in group 2 who received theophylline derivatives bound equivalent amounts of DHA (29.6±2.1 fmol/mg) compared to those not receiving theophylline (25.9±2.7 fmol/mg, $P > 0.1$). Further analysis of these data with respect to sex or age of subjects in the groups yielded no significant difference between males and females ($P > 0.1$) and insignificant correlation of binding at 30 nM DHA with age ($P > 0.05$) in both male and female subjects in all three groups.

When the number of DHA binding sites in group 3 was determined separately for subjects who received or did not receive therapy with corticosteroids, the binding in the 12 subjects receiving steroids was 8.3 ±1.8 fmol/mg, a value not significantly different from the amount of DHA bound in the five subjects not receiving steroids ([5.8±3.9 fmol/mg], $P > 0.2$).

As an additional experiment to determine if a population of asthmatic subjects might have differences in binding of DHA, we measured binding at 0.5 nM DHA in the same 26 control subjects, 22 subjects in group 2, and 17 subjects in group 3 in whom we measured binding at 30 nM DHA (Fig. 2, left panel). Again, subjects in group 1 and 2 showed nearly identical amounts of DHA binding, whereas subjects in group 3 bound significantly ($P < 0.01$) less DHA than those in either of the two other groups.

Although the data presented thus far indicated insignificant differences for binding of the beta adrenergic antagonist DHA in control subjects and asthmatics not receiving adrenergic therapy, it was important to determine if asthmatics might show an altered affinity for a beta adrenergic agonist. We compared 8 asthmatics from group 2 (subjects 23–30) and 10 control subjects (Fig. 3A). Competition for DHA bindings sites by the beta adrenergic agonist (−)-isoproterenol was virtually identical in controls and asthmatics. The dissociation constant for isoproterenol calculated from the results for individual studies of the subjects presented

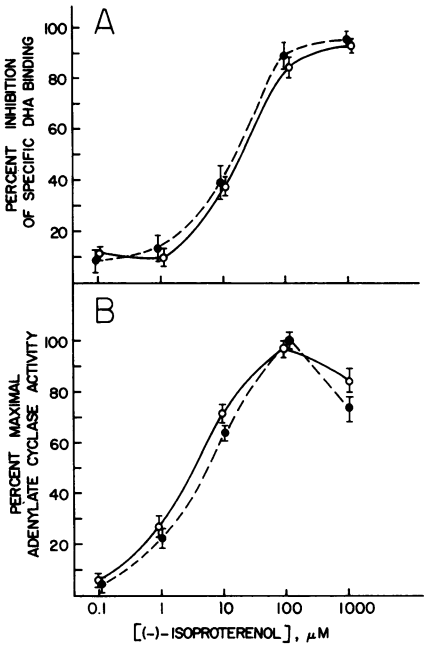


FIGURE 3 (A) Competition for DHA binding sites by (−)-isoproterenol. (B) Stimulation of adenylate cyclase by (−)-isoproterenol in control and asthmatic subjects. DHA binding and adenylate cyclase were examined concurrently as described in Methods. Data shown are mean±SEM from studies on 8 asthmatic (●) and 10 control subjects (○).

in Fig. 3 was 1.06±0.20 μM for control and 1.00±0.20 μM for the asthmatic subjects.

*Adenylate cyclase studies.* As a further measure of changes in beta adrenergic receptors in asthma, we also determined basal and isoproterenol-stimulated adenylate cyclase activity in sonicates prepared from PMN and measured under conditions identical to those used to measure competition of (−)-isoproterenol for DHA binding sites (Fig. 3B). When plotted as percent maximal adenylate cyclase activity, the curves are similar for the two groups, and the $K_{act}$ for (−)-isoproterenol calculated to be 0.16±0.03 for control and 0.19±0.02 for asthmatic subjects ($P > 0.1$). Although the absolute activity of basal adenylate cyclase (Fig. 4) is lower in control than in asthmatic subjects (42.0±4.9 vs. 53.5 ±6.8 pmol cAMP generated/min per mg, this difference is not statistically significant [$P > 0.1$]) and both groups of subjects show about a threefold maximal increase in adenylate cyclase activity in response to isoproterenol and similar responses at each concentration tested. By comparison, the only subject from group 3 (subject 14) whom we tested had a lower basal activity and a blunted response to isoproterenol.

## DISCUSSION

These data indicate that patients with bronchial asthma who are not receiving systemic therapy with adrenergic

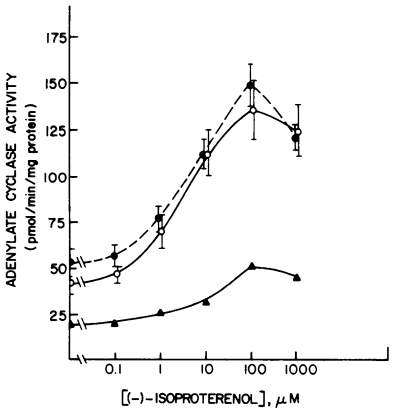


FIGURE 4 Adenylate cyclase activity in PMN particulates from control and asthmatic subjects. Adenylate cyclase activity assayed as described in Methods is plotted for basal activity (intercept on the ordinate) or in response to (−)-isoproterenol. Data shown are mean±SEM for 8 asthmatics on no systemic adrenergic therapy (●), 10 controls (○) and 1 asthmatic (▲) who received terbutaline.

agonist have beta adrenergic receptors on their PMN with the following similar properties compared to control subjects: affinity for the radioligand DHA and for the agonist isoproterenol, number of beta adrenergic receptors, and beta adrenergic stimulated adenylate cyclase activity. In contrast, asthmatics receiving adrenergic agonists systemically have decreased numbers of beta adrenergic binding sites, and decreased isoproterenol-stimulated adenylate cyclase activity in the single subject in whom we measured enzymatic activity. Even though beta adrenergic binding sites are decreased about 70% by adrenergic therapy, the affinity of the remaining binding sites for DHA is similar to the value found in controls and asthmatics not receiving adrenergic agonists. The preferential decrease in number of DHA binding sites in subjects receiving adrenergic agonists is consistent with studies showing that the number, but not the affinity, of radioligand binding sites is decreased after treatment of animals or cells with beta adrenergic agonists (22–26).

Our data offer further evidence that systemic treatment of patients with beta adrenergic agonists appears to "down regulate" receptors for beta adrenergic amines (27). We have recently shown that asthmatics with moderate to severe asthma, comparable to group 3 in this study, have normal binding 1 wk after adrenergic therapy is discontinued (27). This would suggest that it is the adrenergic therapy rather than the severity or duration of asthma, which accounts for the decreased beta adrenergic binding in the asthmatics in group 3 compared to those in group 2 with milder asthma or compared to the control population. Because we have found that asthma in patients withdrawn from adrenergic medication worsened, we did not feel justified in repeating this maneuver with the present subjects (27).

Therefore, because we did not withdraw therapy from subjects in group 3 and because our subjects in group 3 were more ill than those in group 2 and were receiving additional medication (e.g., 0 of 17 subjects in group 3 but 11 of 30 subjects in group 2 received no medication for their asthma), we cannot state unequivocally that therapy with catecholamine agonists, rather than disease severity, is exclusively responsible for the decreased binding in the subjects in group 3. Furthermore, although our findings indicate that treatment with corticosteroids produced no significant increase in the number of beta adrenergic receptors in the asthmatic patients receiving adrenergic agonists, the number of subjects examined was small. More complete studies involving treatment and withdrawal of corticosteroids from normals and asthmatics will be required to ascertain whether such treatment alters properties of beta adrenergic receptors in granulocytes.

It is difficult to be certain that our findings for beta adrenergic receptors in PMN derived from asthmatics necessarily reflect the status of receptors from other tissues involved in the pathophysiology of asthma. However, Conolly and Greenacre (28) have shown that human lymphocytes and human lung parenchyma have $beta_2$-type adrenergic responses to several adrenergic agonists and antagonists and have suggested that peripheral blood lymphocytes may be a reasonable marker tissue for receptors in the lung. Beta adrenergic receptors on human PMN also appear to be of the $beta_2$ type (17). If peripheral blood cells can be considered valid indicators of the state of adrenergic receptors in the bronchopulmonary tree, then our results would suggest that asthmatics may also have normal numbers of pulmonary beta adrenergic receptors. Further proof of this would require direct study of receptors in pulmonary tissue in humans; such studies are not currently feasible.

In an earlier study, Sokol and Beall (29) reported that asthmatics and control subjects had similar numbers of beta adrenergic receptors. In that study the authors identified beta adrenergic receptors by using [$^3$H]epinephrine, a method that was later shown to predominantly represent interaction of oxidized catecholamine derivatives with tissue macromolecules (23). Thus, our results offer more definitive evidence of the similarity of beta adrenergic receptor number in asthmatics and controls.

In addition our results indicate that beta adrenergic receptor affinity and function (i.e., activation of adenylate cyclase) are similar in PMN from asthmatic and control subjects. In this regard, we should note the relationship between the $K_d$ isoproterenol and $K_{act}$ isoproterenol in our subjects. We find that the agonist is more potent in activating adenylate cyclase than in competing with DHA for the receptors. It has been proposed that if binding to receptors and activation of adenylate cyclase are examined under identical con-

ditions, the relationship between $K_d$ and $K_{act}$ offers an index of the efficiency of coupling between beta adrenergic receptors and adenylate cyclase (23). We therefore attempted to perform these two assays concurrently with the same conditions. Our findings indicate that beta adrenergic receptors in the PMN membrane are coupled with high efficiency to adenylate cyclase with a $K_d/K_{act}$ in control subjects of 6.6 and in asthmatics not receiving systemic adrenergic medication of 5.3. These values for PMN particulates are much higher than has been observed for most other tissues with beta adrenergic receptor coupled adenylate cyclase, and are similar to values observed for S49 lymphoma cells, a model system demonstrating highly efficient coupling (23, 30). An alternative way of interpreting the relationship between $K_d$ and $K_{act}$ in PMN is the presence of spare receptors such that only a small fraction of receptors need to be occupied to produce full response.

Our data on asthmatic subjects conflict with two recent reports (31, 32), which showed that lymphocytes from asthmatics have decreased DHA binding even in the absence of adrenergic therapy. Additionally, our finding of statistically insignificant changes in number of PMN beta adrenergic receptor with increasing age contradict the findings of Schocken and Roth (33) who observed a decrease in number of DHA binding sites on lymphocytes as a function of increasing age. Because recent evidence indicating differences in beta adrenergic receptors in lymphocyte subpopulations (10), it is possible that the data in lymphocytes may be explained by differences in distribution of lymphocyte subclasses in asthmatics and older people. Alternatively, we have to consider the possibility that data obtained with PMN may not necessarily apply to other beta adrenergic receptors in the body.

The preliminary findings recently reported by Sano et al. (34) suggest that this theoretical possibility may, in fact, apply. Using a different radioligand ([$^{125}$I]iodohydroxybenzylpindolol), Sano et al. found results identical to ours in granulocytes but noted that lymphocytes showed a decrease in number of beta adrenergic receptors even in asthmatics who did not receive adrenergic agonists. More complete characteriztion of beta adrenergic receptors in other tissues will be necessary to determine the validity of results obtained using various types of blood cells.

With respect to asthma and PMN, Busse and Sosman (35) have reported that intact PMN from asthmatic subjects who have been withdrawn from adrenergic therapy for at least 2 wk have decreased histamine and isoproterenol-stimulated cyclic AMP accumulation (16, 35). Busse and Sosman's results thus indicate that PMN are altered in asthma independent of adrenergic therapy. In reconciling Busse and Sosman's findings and other reports in the literature with those in this study, we suggest that if beta adrenergic hyporesponsiveness occurs in intact leukocytes from patients with bronchial asthma, this decreased response is not attributable to defective beta adrenergic receptor binding as detected in disrupted PMN. In light of recent evidence indicating differences in properties of beta adrenergic receptors in intact and broken cells (30, 36, 37), it may prove fruitful to perform more detailed studies comparing adrenergic receptors and adrenergic response in intact PMN prepared from asthmatic and control subjects.

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