

Measurement of β -Adrenergic Receptors on Lymphocytes in Normal Subjects and Asthmatics in Relation to β -Adrenergic Hyperglycaemic Response and Bronchial Responsiveness

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The numbers of β -adrenergic receptors on lymphocytes in normal subjects and asthmatic patients were measured by the use of [^{125} I]hydroxybenzylpindolol. The numbers of β -adrenergic receptors per lymphocyte in normal subjects, drug-free asthmatics and patients taking β -stimulants were 1146 ± 98 , 845 ± 114 and 582 ± 47 sites/cell (mean $\pm$ SE), respectively. The differences were statistically significant ($P < 0.05$) among these groups, while no statistically significant differences were found in dissociation constants. A 42% decrease in the number of β -adrenergic receptors per lymphocyte after administration of 6 mg/day of terbutaline for 7 days was noted in four volunteers. There was significant correlation ($r = 0.68$, $P < 0.01$) between the number of β -adrenergic receptors per lymphocyte and the percentage increase in blood sugar 20 min after subcutaneous injection of 4 μ g/kg epinephrine. There was also significant correlation ($r = 0.78$, $P < 0.005$) between the number of β -adrenergic receptors per lymphocyte and the respiratory threshold for acetylcholine. These results suggest that β -blockade and bronchial hypersensitivity in asthmatic patients may in part be due to a decreased number of β -adrenergic receptors.

Key words: β -adrenergic receptor; β -adrenergic hyperglycaemic response; bronchial asthma; bronchial responsiveness; lymphocyte.

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CLINICAL ASPECTS

Szentivanyi's hypothesis of β -adrenergic blockade in asthma is supported in the literature. We measured the number of β -adrenergic receptors on lymphocytes in normal subjects and asthmatics to ascertain whether the abnormality of β -adrenergic receptors is present in asthmatic patients. The number of β -adrenergic receptors per lymphocyte in asthmatics was significantly smaller than in normal subjects. Significant correlation was found between the number of β -adrenergic receptors per lymphocyte and the percentage increase in blood sugar after subcutaneous injection of epinephrine. Significant correlation was also found between the number of β -adrenergic receptors per lymphocyte and the respiratory threshold for acetylcholine. These results suggest that β -blockade and bronchial hypersensitivity in asthmatics may in part be due to a decreased number of β -adrenergic receptors.

The hypothesis of β -adrenergic blockade in asthma proposed by Szentivanyi (1) is supported in the literature (2, 3). In recent years, measurement of the density of β -adrenergic receptors on human leukocytes has been carried out by the use of radioligands (4, 5). The objectives of this study were: 1) comparison of

β -adrenergic receptor density per lymphocyte between normal subjects and asthmatics, 2) detection of changes in β -adrenergic receptor density after successive administration of β -stimulants, 3) study of the relationship between the number of β -adrenergic receptors on lymphocytes and β -adrenergic hyperglycaemic

responses to epinephrine, and 4) study of the relationship between the number of receptors and bronchial responsiveness as measured in terms of the respiratory threshold for acetylcholine.

MATERIAL AND METHODS

Subjects

The subjects were 11 normal subjects and 19 asymptomatic asthmatic in- and out-patients at the Dokkyo University Hospital. Their body weights were within $\pm 15\%$ of standard. Normal subjects were all males, 24 to 68 years of age with a mean of 38. Eight of the 19 asthmatic patients were drug-free for at least 2 weeks before study and had no attacks (drug-free group). The drug-free group consisted of six males and two females, 16 to 54 years of age with a mean of 35. Eleven of the 19 asthmatics received β -stimulants regularly (β -stimulant group). The β -stimulant group consisted of six males and five females, 16 to 60 years of age with a mean of 37. Six patients in this group received 5–7.5 mg prednisolone/day. Table 1 summarizes the background factors of the asthmatic patients: age, sex, duration of asthma, type of asthma and daily dosage of prednisolone. No significant difference in age distribution was noted between groups. Subjects were specially chosen so as to avoid any significant difference in age, because the correlation between age and β -adrenergic receptor density is controversial (6, 7).

Lymphocyte preparation

At 10 a.m. 40 ml blood was drawn from the ante-cubital vein of each subject, and heparinized. The subjects had been given no drugs for at least 12 h prior to sampling. The lymphocyte layer was collected from the blood centrifuged on Ficoll-Hypaque density gradient (8), washed three times in phosphate-buffered saline, suspended in incubation buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.4) at a concentration of 7.5×10^6 cells/ml and stored at $-70^\circ C$.

Table 1

Background factors of asthmatic patients

(1) Drug-free group

NO.	Name	Age	Sex	Duration (years)	Type
1	K.N.	16	male	11	atopic
2	K.N.	53	male	7	mixed
3	Y.W.	30	male	26	atopic
4	H.S.	54	male	13	mixed
5	M.T.	23	female	9	atopic
6	T.H.	30	male	9	mixed
7	S.K.	27	male	6	mixed
8	T.S.	50	male	4	mixed
	mean	35		11	

(2) β -stimulant group

NO.	Name	Age	Sex	Duration (years)	Type	Daily dosage of prednisolone
1	H.K.	38	male	3	infectious	
2	T.U.	34	male	31	mixed	
3	I.S.	16	male	3	mixed	
4	T.M.	44	male	22	mixed	
5	Y.U.	34	female	10	mixed	
6	M.M.	25	female	16	mixed	5 mg
7	M.S.	26	male	7	infectious	7.5 mg
8	H.K.	36	male	8	infectious	5 mg
9	F.W.	60	female	7	infectious	7.5 mg
10	M.S.	45	female	7	infectious	5 mg
11	F.S.	45	female	8	infectious	7.5 mg
	mean	37		11		

Binding assay of β -adrenergic receptor

[^{125}I]hydroxybenzylpindolol (IHYP, Amersham) was used as a radioligand, prepared in five concentrations of 0.03–1.29 nM in incubation buffer. Lymphocytes were homogenized by a homogenizer (Kayashima) fitted with a motor-driven teflon paste. A mixture of 200 μ l lymphocyte homogenate, 100 μ l IHYP solution and 100 μ l incubation buffer was kept at room temperature for 60 min and diluted with 3 ml cold incubation buffer to stop the reaction, followed by rapid vacuum filtration through a GF/c glass fibre filter (Whatman). The filter was rapidly washed by vacuum filtration of 8 ml cold incubation buffer, placed in a plastic tube and the radioactivity counted with the Auto Well Gamma System ARC 351 (Aloka). In each experiment, "non-specific" binding to homogenate protein was determined by counting the radioactivity retained on the filter when incubation was performed in the presence of high concentration (1 μ M) ($\pm$) propranolol hydrochloride (Sigma). "Specific" binding was defined as total binding to homogenate protein minus

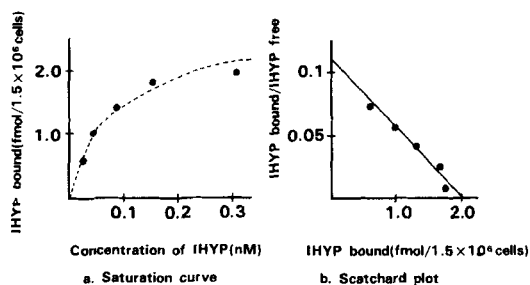


Fig. 1. Saturation curve of IHYP specific binding to lymphocyte homogenate protein and its analysed plot by the Scatchard method.

non-specific binding. The saturation curve was plotted from the concentrations of IHYP and the specific binding readings (Fig. 1a), which were analysed by the Scatchard method (9) (Fig. 1b), and the β -adrenergic receptor density and affinity for IHYP were calculated. Experiments were performed in triplicate.

Administration of terbutaline

Four normal volunteers received 2 mg terbutaline sulphate 3 times a day after meals for 7 days. Forty ml blood was drawn from each subject before and after administration of terbutaline, heparinized, and β -adrenergic receptors per lymphocyte were counted by the method mentioned above.

Hyperglycaemic response to epinephrine injection

The increase in blood sugar after subcutaneous injection of epinephrine was studied in six normal subjects and eight asthmatics. At mid-day fasting blood samples were collected followed immediately by s.c. injection of 4 μ g/kg of epinephrine hydrochloride (Bosmin®, Dai-ichi Pharm.). Twenty minutes after injection, blood was drawn again and blood sugar measured by Hoffman's method.

Acetylcholine inhalation test

An acetylcholine inhalation test was performed on eight normal subjects and 10 asthmatics. All drugs were withheld, except in those subjects already receiving corticosteroids, at least 12 h

before the test. Subjects inhaled aerosolized acetylcholine solution for 1 min, the concentration of which was serially doubled from 78 to 20,000 μ g/ml. The concentration of acetylcholine inducing a 50% increase in respiratory resistance was referred to as the respiratory threshold for acetylcholine. The respiratory resistance was measured by the oscillation method (10) (3 Hz) using a respiratory resistance meter MZR 4000 (Nihon Koden).

Statistical test

The data were assessed by t-test for the significance of difference, and the differences were considered statistically significant when the levels of confidence were over 95%.

RESULTS

Number of β -adrenergic receptors per lymphocyte

Fig. 2 shows the number of β -adrenergic receptors per lymphocyte in each subject. The numbers of β -adrenergic receptors per lymphocyte in normal subjects, drug-free group and β -stimulant group were 1146 ± 98 , 845 ± 114 and 582 ± 47 sites/cell (mean $\pm$ SE), respectively. The differences were statistically significant ($P < 0.05$).

In the β -stimulant group the numbers of β -adrenergic receptors per lymphocyte were compared between six subjects on corticosteroid therapy and five receiving no corticosteroids, and were found to be 551 ± 60 and 619 ± 71 sites/cell (mean $\pm$ SE), respectively.

There was no significant difference. The amount of β -stimulants was not different either.

Dissociation constant

The dissociation constants of normal subjects, drug-free group and β -stimulant group were $(1.54 \pm 0.34) \times 10^{-10}$, $(1.18 \pm 0.37) \times 10^{-10}$ and $(1.08 \pm 0.37) \times 10^{-10}$ M (mean $\pm$ SE), respectively. In the β -stimulant group, dissociation constant in subjects receiving corticosteroids was $(1.30 \pm 0.40) \times 10^{-10}$ and $(0.81 \pm 0.20) \times 10^{-10}$

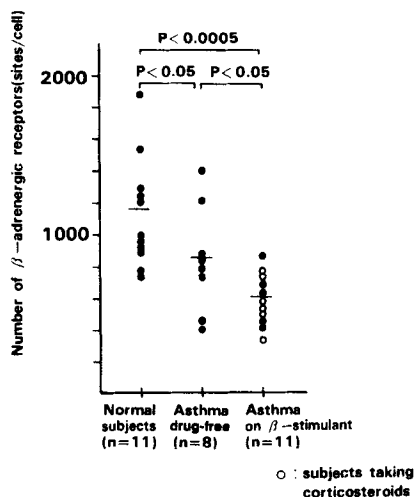


Fig. 2. Number of β -adrenergic receptors per lymphocyte in each subject.

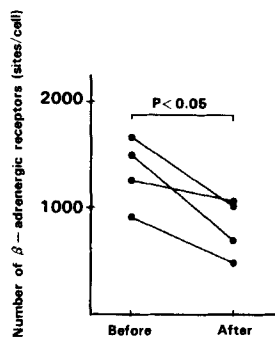


Fig. 3. Number of β -adrenergic receptors per lymphocyte in each subject counted 1 week before and after administration of 6 mg terbutaline/day.

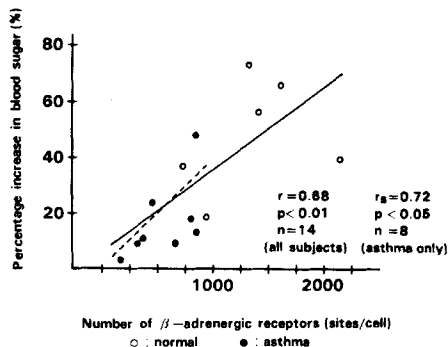


Fig. 4. Relationship between number of β -adrenergic receptors per lymphocyte and percentage increase in blood sugar 20 min after subcutaneous injection of 4 $\mu\text{g/kg}$ epinephrine.

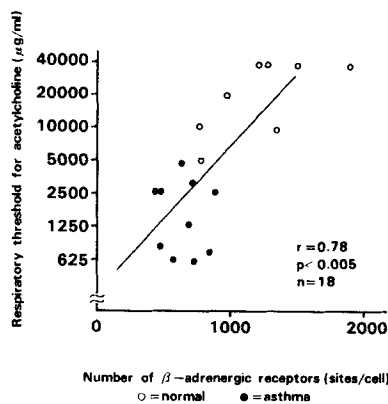


Fig. 5. Relationship between number of β -adrenergic receptors per lymphocyte and bronchial responsiveness, expressed by the respiratory threshold for acetylcholine.

M, respectively. No statistically significant difference was noted between these groups.

Effects of terbutaline on number of β -adrenergic receptors

The number of β -adrenergic receptors per lymphocyte in each subject 1 week before and 1 week after administration of 6 mg terbutaline/day was 1348 ± 139 and 782 ± 100 sites/cell (mean $\pm$ SE), respectively (Fig. 3). The number diminished significantly ($P < 0.05$) after terbutaline.

Relationship between number of β -adrenergic receptors and hyperglycaemic response to epinephrine

Fig. 4 shows the relationship between the number of β -adrenergic receptors per lymphocyte and percentage increase in blood sugar 20 min after subcutaneous injection of epinephrine. When data of normal subjects and asthmatics were considered together, a significant correlation was noted ($r = 0.68$, $P < 0.01$). The solid lines shows the regression line calculated by the least square method. When only data of asthmatics were analysed, Spearman's rank correlation coefficient (r_s) was 0.72, and was statistically significant ($P < 0.05$). The dotted line shows the regression line.

Relationship between number of β -adrenergic receptors and respiratory threshold for acetylcholine

Fig. 5 shows the relationship between the number of β -adrenergic receptors per lymphocyte and the respiratory threshold for acetylcholine. Subjects having a threshold of more than 20,000 $\mu\text{g}/\text{ml}$ were regarded as having a threshold of 40,000 $\mu\text{g}/\text{ml}$. When data of all subjects were considered together, a significant correlation ($r = 0.78$, $P < 0.005$) was noted between them.

However, when data of asthmatics only were analysed, no significant correlation was found.

DISCUSSION

We measured the number of β -adrenergic receptors per lymphocyte in normal subjects and asthmatic patients, using IHYP, and found a significantly smaller number in asthmatics than in normal subjects. In asthmatic patients, those on β -stimulants had a smaller number of β -adrenergic receptors than those in the drug-free group. Brooks et al. (11), who used [^3H] dihydroalprenolol (DHA), reported that the number of β -adrenergic receptors per lymphocyte in asthmatics not taking β -stimulants was smaller than in normal subjects. Szentivanyi et al. (12) reported a similar result. However, Galant et al. (13) found no significant difference in the number of β -adrenergic receptors per polymorphonuclear leukocyte between normal subjects and asthmatics not taking β -stimulants. Sano et al. (14) measured the number of β -adrenergic receptors per lymphocyte and granulocyte in normal subjects and asthmatics, and found a significant difference in lymphocytes, but no difference in granulocytes. It is reported that the increases in blood sugar, plasma cyclic AMP after injection of epinephrine, and cyclic AMP response of lymphocytes to isoproterenol in drug-free asthmatics are lower than in normal subjects (2, 15, 16). Based on our results and the above reports, we are inclined to think that β -receptors are abnormal in asthma and are not affected by β -stimulants.

We found the same marked decrease in the number of β -adrenergic receptors per lymphocyte in asthmatics on β -stimulants, as in the previous reports (13, 14). In order to know by how much β -stimulants decrease the number of β -adrenergic receptors, we administered 6 mg of terbutaline/day for 7 days and found that this small dose of terbutaline induced a 42% decrease in the number of β -adrenergic receptors per lymphocyte. Galant et al. (17) demonstrated an 80% decrease in the number of β -adrenergic receptors per polymorphonuclear leukocyte after administration of 10 mg terbutaline/day for 7 days. Sano et al. (18) reported an 85% decrease in the number of β -adrenergic receptors per lymphocyte after administration of 15 mg terbutaline/day for 7 days.

It may therefore be that the decrease in the number of β -adrenergic receptors per leukocyte in asthmatics on β -stimulants was ascribable not only to the asthma itself but also to β -stimulants.

In the β -stimulant group, we noticed no difference in the number of β -adrenergic receptors between patients on corticosteroids and patients receiving no corticosteroids. Galant et al. (13) reported the same result with polymorphonuclear leukocytes. After using corticosteroids, Mano et al. (19), Fraser et al. (20) and Davies et al. (21) noticed a significant increase in β -adrenergic receptor density in rat lungs, cultured human lungs and leukocytes. Judging from our results and other reports, the number of β -adrenergic receptors in asthmatics receiving corticosteroids regularly may decrease when they stop receiving corticosteroids.

In accordance with Galant et al. (13) we found no difference in dissociation constants among the groups. However, we found that the dissociation constant in patients taking corticosteroids was slightly, but not significantly, larger than in patients not on corticosteroids. In fact, Mano et al. (19) noticed that the dissociation constant of rat lung β -adrenergic receptors tended to increase after injection of hydrocortisone for 9 days, and Davies et al. (21) noticed a marked increase in the dissociation constant of polymorphonuclear leukocyte β -adrenergic receptors 4 h after oral administration of 100 mg cortisone acetate.

Next, we studied the relationship between the number of β -adrenergic receptors per lymphocyte and the percentage increase in blood sugar after subcutaneous injection of epinephrine, and found a significant correlation. A comparative study revealed that β -adrenergic receptors on leukocytes are of the β_2 -type (4, 5), and an increase in blood sugar after injection of epinephrine also represents a β_2 -adrenergic response, mostly in liver cells (22). Wessels et al. (23) and Mukherjee et al. (24) reported a close relationship between the β -adrenergic receptor density and adenylate cyclase activity in frog erythrocytes. Thus, it is conceivable that the decrease in the number of β -adrenergic receptors in liver cells caused a decrease in hyperglycaemic response to epinephrine and that the abnormality of β -adrenergic receptors on lymphocytes is related to liver cell disorders.

Radioligands used in the binding assay of β -adrenergic receptors are β -antagonists, DHA IHYP, and β -agonist, [^{3} H]dihydroisoproterenol (DHI). Wessels et al. (23) reported a discrepancy between DHA and DHI in the numbers of β -adrenergic receptors. From the thermodynamic standpoint Weiland et al. (25) reported a difference in binding to β -adrenergic receptors between agonist and antagonist. Despite many problems, the significant correlation between the number of β -adrenergic receptors per lymphocyte and the percentage increase in blood sugar after injection of epinephrine shows the suitability of lymphocytes as a whole body model.

Lastly, we found a significant correlation between the number of β -adrenergic receptors per lymphocyte and the responsiveness of bronchi expressed by the respiratory threshold for acetylcholine. Makino et al. (26) reported a significant correlation between the respiratory threshold for acetylcholine and the percentage increase in blood sugar after injection of epinephrine. Apold et al. (15) also reported a significant correlation between the respiratory threshold for histamine and the percentage increase in plasma cyclic AMP after injection of epinephrine. Our reports are consistent with the reports of Makino and Apold. All these findings

suggest that bronchial hypersensitivity in asthmatics may in part be due to β -blockade, although the abnormality of β -adrenergic receptors in the lung was not studied.

Thus we found: 1) a decrease in the number of β -adrenergic receptors on lymphocytes in asthmatics, 2) a decrease in the number of β -adrenergic receptors after administration of β -stimulants, 3) a significant correlation between the number of β -adrenergic receptors per lymphocyte and the percentage increase in blood sugar after injection of epinephrine, and 4) a significant correlation between the number of β -adrenergic receptors and the respiratory threshold for acetylcholine. We therefore conclude that β -blockade and bronchial hypersensitivity in asthmatic patients may in part be due to the decreased number of β -adrenergic receptors, and that subsensitivity to β -stimulants (27) after administration of β -stimulants may be in part due to a decrease in the number of β -adrenergic receptors.

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