

Relationships between Responsiveness of the Bronchi to Acetylcholine and Cyclic AMP Response of Lymphocytes to Beta₁- and Beta₂-Adrenergic Receptor Stimulation in Patients with Asthma

SOHEI MAKINO, RYOSUKE IKEMORI, TAKESHI FUKUDA and SHINJI MOTOJIMA

Dept. of Medicine and Clinical Immunology School of Medicine, University of Dokkyo Tochigi, Japan

Decreased response of beta-adrenergic receptor has been considered to be one of the causes of increased responsiveness of the bronchi in asthma. Since beta-adrenergic receptor has two subtypes, beta₁ and beta₂, and the bronchodilating effect of beta stimulants is mediated by beta₂-receptor, responsiveness of the bronchi is expected to correlate to the cyclic AMP response of lymphocytes to a beta₂-stimulant. Responsiveness of the bronchi was expressed as respiratory threshold to acetylcholine (RT-Ach), which was the minimal concentration of acetylcholine solution to cause an initial decrease of FEV₁ of more than 20% of the baseline value. Beta₁- and beta₂-responses were expressed as the increments of cyclic AMP content of 10⁶ lymphocytes incubated with norepinephrine (beta₁-stimulant) and salbutamol (beta₂-stimulant).

RT-Ach showed a significant correlation with the beta₂-cyclic AMP response of lymphocytes, but not with the beta₁-response among patients with asthma. Sixteen symptomatic patients on continuous beta-stimulants showed lower RT-Ach value and diminished beta₂-receptor activity of lymphocytes compared with 14 patients in remission. These results suggest that selective beta₂-adrenergic blockade may be one of the causes of bronchial hypersensitivity in asthma, though it should be noted that in this study beta-adrenergic responses were examined in lymphocytes and were compared with the responsiveness of the bronchi. Possible beta-receptor subsensitivity induced by administration of beta-stimulants is discussed.

Key words: asthma; beta-adrenergic receptors; adenosine cyclic monophosphate; inhalation provocation test.

Accepted for publication 11 August 1982

CLINICAL ASPECTS

Bronchial asthma is a common disease, and although we have many drugs for treating bronchoconstriction in asthmatic attacks no cure has been found.

Hypersensitivity of the bronchi to various stimuli is one of the main characteristics of bronchial asthma. Although the etiology of this hypersensitivity is not known, decreased beta-adrenergic response in bronchi has been proposed as one of the mechanisms. Beta-adrenergic receptors have two subtypes, beta₁ and beta₂. Beta₂-receptors mediate bronchodilating effect of catecholamines, while beta₁-receptors mediate cardiac stimulation. If beta-adrenergic blockade were the cause of bronchial hypersensitivity, the abnormality would be in beta₂-receptors, but not in beta₁-receptors.

We found a low but statistically significant correlation between bronchial hypersensitivity to inhaled acetylcholine and cyclic AMP response of lymphocytes to beta₂-stimulant, salbutamol, in patients with asthma. Though the organs examined were different, these observations suggest that the abnormality of beta₂-receptors may be one of the causes of bronchial hypersensitivity in asthma.

Decreased beta-adrenergic response has been proposed as one of the causes of hypersensitivity of the bronchi in bronchial asthma (22). It has been reported that responsiveness of the bronchi to parasympathomimetic agents or histamine showed inverse correlation with beta-adrenergic responses as measured by the increase of blood level of cyclic AMP, glucose and lactic acid, and eosinopenia induced by the injections of beta-stimulants in patients with asthma (1, 14). Such observations suggest that reduced bronchodilating effects of endogenous catecholamines enhances bronchoconstriction induced by various stimuli. Beta-adrenergic receptors have two subtypes, β_1 and β_2 . In the lung bronchodilation is mediated by β_2 -receptor (16, 18) so it is possible that abnormality of beta-responses in asthma is restricted to β_2 -response.

Norepinephrine and salbutamol stimulate predominantly β_1 - and β_2 -receptors, respectively (15). We measured responsiveness of the bronchi to acetylcholine and cyclic AMP (cAMP) production of lymphocytes by β_1 - and β_2 -agonists in asthmatic patients in remission and with moderate symptoms.

There have been no reports which definitely showed that the bronchi of patients with asthma were less reactive to β_2 -adrenergic bronchodilation. It is thought that if β_2 -adrenergic response in lymphocytes shows an inverse correlation to responsiveness of the bronchi to acetylcholine and β_1 -response does not, then it is possible that selective β_2 -adrenergic subsensitivity is one of the causes of bronchial hypersensitivity in asthma.

The aims of this study are to find out whether or not responsiveness of the bronchi is correlated with the β_2 -response of lymphocytes and to investigate the hypothesis of selective β_2 -adrenergic subsensitivity in asthma.

SUBJECTS AND METHODS

Subjects

The study comprised 30 patients with asthma (21 male and 9 female). Their age ranged from 17 to 50 years; mean 30.3. Fourteen of the 30

patients were symptomatic and were kept on beta-stimulants and theophyllines. The remaining 14 patients were asymptomatic and were drug-free at least 2 weeks before tests (9).

Preparation of cells and measurement of cyclic AMP response

Venous blood (40 ml) was drawn from the antecubital vein between 9 and 10 a.m. The subjects did not take medications or beverages containing caffeine for at least 12 h before blood sampling. Lymphocytes were prepared by density gradient centrifugation in Ficoll-Hypaque as described by Boyum (2). The purified preparations contained more than 90% lymphocytes, the remaining cells being granulocytes or monocytes. The lymphocytes were resuspended in a medium consisting of NaCl, 120 mM; KCl, 5 mM; CaCl_2 , 0.6 mM; MgCl_2 , 1 mM; tris (hydroxymethyl) aminomethane, 25 mM and human serum albumin, 0.03% adjusted to pH 7.4 (6). The incubations were carried out in 0.5 ml vol in duplicate at 37°C. The cells, $3 \times 10^5/\text{mm}^3$, were preincubated for 10 min. Theophylline and norepinephrine or salbutamol were added as required, and the incubation was allowed to continue for an additional 15 min. The cells were then centrifuged for 3 min at 1,500 g, the supernatants were discarded, and the cell buttons were frozen in dry-ice acetone and thawed in 0.5 ml distilled water. After one additional freezing, the tubes were immersed in boiling water for 5 min and centrifuged for 15 min at 2,000 g to remove protein. The supernatants were collected, frozen, and dried for storage. Cyclic AMP was measured by the binding protein method with a commercial kit (cyclic AMP assay kit, Radiochemical Center, Amersham, U.K.) (20).

Cyclic AMP responses of lymphocytes to norepinephrine (l-norepinephrine bitartrate, Sigma Chemical Co.) and salbutamol (salbutamol sulfate, Japan Glaxo Co.), 10^{-5} M and 10^{-6} M, were measured in the presence of 5×10^{-4} M of theophylline (Daichi Kagaku, Tokyo, Japan) to enhance the effect of beta-stimulants by inhibiting phosphodiesterase. This concentration of theophylline did not change the content of

Table 1
Level of cyclic AMP in lymphocytes from patients with asthma

Treatment	Mean and SD of cAMP level (p mol/ 10^6 lymphocytes)			
	Total <i>n</i> = 30	Symptomatic (with beta-stimulants) <i>n</i> = 14	Asymptomatic (without beta-stimulants) <i>n</i> = 16	
None	4.5 $\pm$ 4.8	4.3 $\pm$ 6.2	4.8 $\pm$ 3.1	NS*
Theophylline 5×10^{-3} M	3.8 $\pm$ 2.6	3.6 $\pm$ 1.8	4.0 $\pm$ 3.2	NS
Norepinephrine 10^{-3} M	14.0 $\pm$ 5.8	12.7 $\pm$ 6.2	15.1 $\pm$ 7.0	NS
Norepinephrine 10^{-5} M	11.5 $\pm$ 5.9	9.1 $\pm$ 3.6	13.7 $\pm$ 7.3	<i>P</i> < 0.05
Salbutamol 10^{-3} M	8.6 $\pm$ 4.8	6.3 $\pm$ 2.0	10.6 $\pm$ 6.3	<i>P</i> < 0.05
Salbutamol 10^{-5} M	8.0 $\pm$ 4.5	5.5 $\pm$ 2.4	10.1 $\pm$ 5.7	<i>P</i> < 0.01

* Difference between symptomatic and asymptomatic patients, NS: not significant.

cAMP (Table 1). The cAMP level with 5×10^{-4} M theophylline served as the baseline value.

Blood samplings for cAMP responses were done within 2 weeks prior to acetylcholine inhalation tests. No subjects showed apparent changes of asthmatic symptoms between blood sampling and inhalation test.

Acetylcholine inhalation test (14, 23)

Subjects took no drugs at least 12 h before test and showed no asthmatic symptoms at the time of test. They inhaled 14 l of aerosols of an acetylcholine solution which was stored in a drum, and FEV₁ was measured immediately after the inhalation. Aerosols were generated with a Vaponephrine plastic nebulizer with 5 l/min of compressed air. The concentration of acetylcholine solutions was serially doubled from 98 to 25000 μ g/ml. The inhalation started with the lowest concentration of acetylcholine solution and progressed to the higher concentration, until FEV₁ decreased by more than 20% of the baseline value which was measured before the test.

The concentration of acetylcholine solution which showed an initial decrease of FEV₁ by more than 20% of the baseline value was taken as the respiratory threshold to acetylcholine (RT-Ach). RT-Ach is considered to express the responsiveness of the bronchi.

RESULTS

Table 1 and Fig. 1 show cAMP response of lymphocytes to 10^{-3} M and 10^{-5} M of norepinephrine and salbutamol. Both of the drugs increased cAMP level. Preliminary experiments

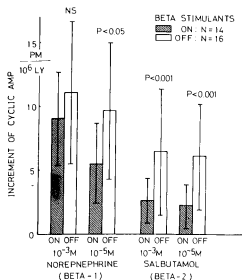


Fig. 1. Comparison of cAMP response of lymphocytes to norepinephrine and salbutamol between symptomatic (with beta-stimulants) and asymptomatic (without beta-stimulants) patients with asthma.

Each column and bar show mean and SD of increment of cAMP in 10^6 lymphocytes with incubation of norepinephrine (beta₁-stimulant) and salbutamol (beta₂-stimulant).

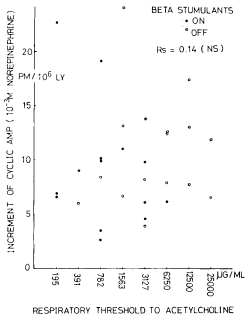


Fig. 2. Relationship between responsiveness of the bronchi to acetylcholine (RT-Ach) and cAMP response of lymphocytes to 10^{-3} M norepinephrine (β_1 -stimulant) among patients with asthma.

Spearman rank correlation coefficient (R_s) was 0.14 and was not statistically significant. Closed circles: patients on beta-stimulants. Open circles: patients not on drugs.

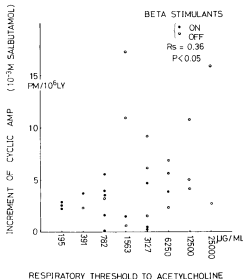


Fig. 3. Relationship between responsiveness of the bronchi (RT-Ach) and cAMP response of lymphocytes to salbutamol (β_2 -stimulant) among patients with asthma. Spearman rank correlation coefficient (R_s) was 0.36 and was statistically significant ($P < 0.05$).

Table 2

Correlation coefficients between respiratory threshold to acetylcholine and cyclic AMP response of lymphocytes to β_1 - and β_2 -stimulants

Treatment of lymphocytes	Spearman rank correlation coefficients*	
Norepinephrine 10^{-3} M	0.14	NS**
Norepinephrine 10^{-5} M	0.20	NS
Salbutamol 10^{-3} M	0.36	$P < 0.05$
Salbutamol 10^{-5} M	0.40	$P < 0.05$

* Number of subjects was 30; ** NS: not significant.

showed that the cAMP response of the two β -stimulants was maximal at 10^{-3} M, since no further increase of cAMP production was observed at 10^{-2} M.

The increments of the cAMP level of lymphocytes with norepinephrine and salbutamol were compared between symptomatic and asymptomatic patients. The cAMP increment with 10^{-3} M and 10^{-5} M salbutamol was lower in symptomatic patients compared with that in asymptomatic patients, while the increment with 10^{-3} M norepinephrine was not significantly different between symptomatic and asymptomatic patients.

Thus, symptomatic patients showed a markedly diminished β_2 -receptor response in lymphocytes compared with asymptomatic patients, but the β_1 -receptor response in lymphocytes was not so different between symptomatic and asymptomatic patients as was the β_2 -receptor response.

Table 2 and Figs. 2 and 3 show relationships between RT-Ach and cAMP response of lymphocytes to β_1 - and β_2 -stimulants. No significant correlation was observed between RT-Ach and cAMP response of lymphocytes to β_1 -stimulant (norepinephrine) while RT-Ach showed low but statistically significant correlation to cAMP response to β_2 -stimulant (salbutamol). Thus, patients with a more increased bronchial sensitivity tend to show a lower β_2 -response of lymphocytes. As seen in Figs. 2 and 3, RT-Ach of symptomatic patients were lower than those of asymptomatic patients. The geometric mean of RT-Ach in symptomatic patients

was 905 $\mu\text{g/ml}$ and in asymptomatic patients 4232 $\mu\text{g/ml}$. The difference was statistically significant ($P < 0.01$).

DISCUSSION

RT-Ach expressing responsiveness of the bronchi showed a statistically significant correlation with the β_2 cAMP response in lymphocytes in patients with asthma, but not with the β_1 -response. In a previous report, we found a significant inverse correlation between RT-Ach and β_2 -adrenergic responses to infused epinephrine such as hyperglycemia, hyperlactacidemia and eosinopenia in asthma (14). These results suggest that selective β_2 -adrenergic blockade may be one of the causes of bronchial hypersensitivity in asthma, though it should be noted that in this study the cells examined were lymphocytes and the concentrations of sympathomimetic agents far exceeded physiologic and therapeutic levels.

In this study subjects consisted of symptomatic and asymptomatic patients. Symptomatic patients needed to take beta-stimulants to control asthmatic symptoms, and asymptomatic patients were in remission and were not taking any drugs. Symptomatic patients showed lower RT-Ach and also decreased cAMP response to β_2 -stimulants compared with asymptomatic patients. β_1 cAMP response was not different at 10^{-3} M of norepinephrine and slightly lowered at 10^{-5} M in symptomatic patients compared with asymptomatic patients. These observations suggest that patients with more severe symptoms tend to show increased responsiveness of the bronchi and decreased β_2 -response in lymphocytes, confirming previous reports (3, 10, 13). Interestingly, the β_1 -response was not much different between symptomatic and asymptomatic patients.

Continuous administration of oral beta-stimulants diminishes such beta-responses as hyperglycemic, hyperlactacidemic, cardiac and cAMP, and the number of beta-receptors in leukocytes (9, 11, 21). Subsensitization induced by beta-stimulant administration disappears 2 weeks after cessation of the drugs (9). Since symptomatic patients had been kept on beta-

stimulants in this study, it is considered that the β_2 -subsensitization in lymphocytes observed in symptomatic subjects is in part induced by the administration of beta-stimulants.

However, the administration of beta-stimulants is not considered to be the only cause of β_2 -subsensitization observed in symptomatic patients, since cAMP response to beta-stimulants and the number of beta-receptors in lymphocytes of asthmatic patients diminished even among patients in remission who are not taking beta-stimulants (4, 12, 19).

The design of this study did not allow us to quantitate how much the administration of beta-stimulants contributed to subsensitization to cAMP response. Further well-designed study will be needed to elucidate the intrinsic abnormality of beta-receptors in symptomatic patients.

The correlation coefficient between RT-Ach and cAMP response to β_2 -stimulant was statistically significant but its value was low. This result suggests that bronchial hypersensitivity is related not only to the diminished β_2 -adrenergic response, but also to many other factors. In fact abnormalities of histamine H_2 receptors (5), alpha-adrenergic receptors (8), and slow-adapting irritant receptors (17) have been reported in asthma. Responsiveness of the bronchi is also influenced by the caliber of the airways before the test.

REFERENCES

1. Apold, J., & Aksnes, L.: Correlation between increased bronchial responsiveness to histamine and diminished plasma cyclic adenosine monophosphate response after epinephrine in asthmatic children. Diminished plasma cyclic adenosine monophosphate response after epinephrine in moderate childhood asthma. *J. Allergy Clin. Immunol.* 59, 343-347, 1977.
2. Boyum, A.: Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.* 97 (Suppl. 2): 77, 1968.
3. Brooks, S. M., McGowan, K., Bernstein, I. L., Altenau, P., & Peagler, J.: Relationship between numbers of beta adrenergic receptors in lymphocytes and disease severity in asthma. *J. Allergy Clin. Immunol.* 63, 401-406, 1979.
4. Busse, W. W.: Decreased granulocyte response to isoproterenol in asthma during upper respiratory infections. *Am. Rev. Respir. Dis.* 115, 783-791, 1977.

5. Busse, W. W. & Sosman, J.: Decreased H₂-histamine response of granulocytes of asthmatic patients. *J. Clin. Invest.* 59, 1080-1087, 1977.
6. Gillespie, E., Balentine, M. D. & Lichtenstein, L. M.: Cyclic AMP metabolism in asthma: Studies with leukocytes and lymphocytes. *J. Allergy Clin. Immunol.* 53, 27-33, 1974.
7. Hargreave, F. E., Ryan, G., Thomson, N. C., O'Byrne, P. M., Ratimer, K., Juniper, E. F. & Dolovich, J.: Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance. *J. Allergy Clin. Immunol.* 68, 347-355, 1981.
8. Henderson, W. R., Shelhamer, J. H., Reingold, D. B., Smith, I. J., Evans, R. & Kaliner, M.: Alpha-adrenergic hyperresponsiveness in asthma. Analysis of vascular and pupillary responses. *N. Engl. J. Med.* 300, 642-647, 1979.
9. Jenne, J. W., Chick, T. W., Strickland, R. D. & Wall, F. J.: Subsensitivity of beta response during therapy with a long-acting beta-2 preparation. *J. Allergy Clin. Immunol.* 59, 383-390, 1977.
10. Juniper, E. F., Frith, P. A. & Hargreave, F. E.: Airway responsiveness to histamine and methacholine: relationship to minimum treatment to control symptoms of asthma. *Thorax* 36, 575-579, 1981.
11. Kalisker, A., Nelson, H. E. & Middleton, E., Jr.: Drug-induced changes of adenylate cyclase activity in cells from asthmatic and nonasthmatic subjects. *J. Allergy Clin. Immunol.* 60, 259-265, 1977.
12. Lockett, S. D., Jr., Glennon, J. A. & Reed, C. E.: Comparison of some metabolic responses in normal and asthmatic subjects to epinephrine and glucagon. *J. Allergy* 40, 349-364, 1967.
13. Makino, S.: Clinical significance of bronchial sensitivity to acetylcholine, and histamine in asthma. *J. Allergy* 38, 127-142, 1966.
14. Makino, S., Ouellette, J. J., Reed, C. E. & Fishel, C.: Correlation between increased bronchial response to acetylcholine and diminished metabolic and eosinopenic responses to epinephrine in asthma. *J. Allergy* 46, 178-189, 1970.
15. Makino, S., Ikemori, R., Kashima, T. & Fukuda, T.: Comparison of cyclic adenosine monophosphate response of lymphocytes in normal and asthmatic subjects to norepinephrine and salbutamol. *J. Allergy Clin. Immunol.* 59, 348-352, 1977.
16. Minneman, K. & Molinoff, P. B.: Classification and quantitation of β -adrenergic receptor subtype. *Biochem. Pharmacol.* 29, 1317-1323, 1980.
17. Nadel, J. A.: Role of vagus nerves and airway epithelial damage in regulation of airway smooth muscle, mucus secretion and breathing. In Oehling, A. (ed.): *Advances in allergology and applied immunology*, pp. 309-314. Pergamon Press, Oxford, 1979.
18. Ryo, Y. Y. & Townley, R. G.: Comparison of respiratory and cardiovascular effects of isoproterenol, propranolol, and procaterol in asthmatic and normal subjects. *J. Allergy Clin. Immunol.* 57, 12-24, 1976.
19. Sano, T., Ruprecht, H., Mano, K., Begley, M., Bewtra, A. & Townley, R.: Leukocyte beta-adrenergic receptor assay in normals and asthmatics. *Clin. Res.* 27, 403A, 1979.
20. Steiner, A. L., Pagliara, A. S., Chase, L. R. & Kipnis, D. M.: Radioimmunoassay for cyclic nucleotides. II. Adenosine 3', 5'-monophosphate and guanosine 3', 5'-monophosphate in mammalian tissues and body fluids. *J. Biol. Chem.* 247, 1114-1120, 1972.
21. Svedmyr, N. L. V., Larsson, S. A. & Thiringer, G. K.: Development of "resistance" in beta-adrenergic receptors of asthmatic patients. *Chest* 69, 479-483, 1976.
22. Szentivanyi, A.: The beta-adrenergic theory of the atopic abnormality in bronchial asthma. *J. Allergy* 42, 203-232, 1968.
23. Tiffeneau, R.: L'hyperexcitabilité acetylcholinique du poumon. Critère physio-pharmacodynamique de la maladie asthmatique. *Presse Med.* 63, 227-230, 1955.

Address:

Dr. Sohei Makino, M.D.
Dept. of Medicine and Clinical Immunology
School of Medicine, University of Dokkyo
Mibu, Tochigi-ken 321-02
Japan

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.