

Fenoterol, a selective β_2 -adrenergic agonist, and inhibition of IgE-mediated basophil histamine release

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The present study was undertaken to evaluate the effect of fenoterol, a selective β_2 -adrenergic agonist, on basophil histamine release. Fenoterol at 10^{-7} to 10^{-3} M did not inhibit the release of histamine induced by *Dermatophagoides farinae* extract (D.f.) from leukocytes from allergic patients sensitive to mite. Similarly, there was no suppression of histamine release induced by anti-IgE and formyl-methionyl-leucyl-phenylalanine under the influence of fenoterol. Fenoterol caused a slight inhibition of the calcium ionophore A23187-induced histamine release at 10^{-3} M with % inhibition of 11.8 ± 2.4 ($\bar{x} \pm \text{SEM}$, $P < 0.05$). There was no synergism between fenoterol and theophylline in inhibiting D.f.-induced histamine release. Fenoterol did not suppress the release of histamine induced by antigen at low as well as high levels of release. Based on the data on the effect of fenoterol on IgE-mediated histamine release, it was concluded that in contrast to a human lung mast cell system, the beta-adrenergic receptor-adenylate cyclase system is not a control mechanism in IgE-mediated basophil histamine release.

Key words: basophils; cyclic 3',5'-adenosine monophosphate; *Dermatophagoides farinae*; fenoterol; histamine release.

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IgE-mediated histamine release from mast cells and basophils is an *in vitro* model of immediate hypersensitivity. This reaction by chopped lung fragments and human lung mast cells is inhibited by beta-adrenergic agents which raise intracellular cyclic 3',5'-adenosine monophosphate (cAMP) through interaction with beta-adrenergic receptors (1-4, 9-11). On the other hand, in a human basophil system, concentrations of isoproterenol required to suppress IgE-mediated histamine release are relatively high (5). This finding raises a question about an idea that the beta-adrenergic receptor-adenylate cyclase system is an important control mechanism in IgE-mediated basophil histamine release. Therefore, we examined the effect of fenoterol, a selective β_2 -adrenergic agonist, on basophil histamine release.

MATERIAL AND METHODS

Material

Piperazine-N,N'-bis [2-ethanesulfonic acid] (Pipes) and formyl-methionyl-leucyl-phenylalanine (FMLP) were purchased from the Sigma Chemical Co., St. Louis, MO., USA. The calcium ionophore A23187 was from Calbiochem-Behring Corp., La Jolla, CA, USA, theophylline from Wako Pure Chemical Industries, Osaka, Japan, anti-human IgE from Medical Biological Laboratory, Nagoya, Japan. Lyophilized extract of *Dermatophagoides farinae* (D.f.) was generously provided by Torii Pharmaceutical Co., Tokyo, Japan. Although it was a crude allergen, it did not cause non-specific histamine release from basophils obtained from healthy donors not sensitive to D.f. Fenoterol

was kindly donated by Boehringer-Ingelheim, Kawanishi, Japan.

Preparation of leukocytes

Venous blood was drawn by venipuncture from seven patients with bronchial asthma, one with allergic rhinitis, four with atopic dermatitis and four healthy donors, and allowed to sediment at room temperature for 90 min with 2.5 ml of 6% dextran-2.8% glucose solution (6% dextran in saline, Midorijuji Pharmaceutical Co., Osaka, Japan) per 10 ml of blood. The plasma-leukocyte layer was transferred to a new tube. After centrifugation at $400 \times g$ at 4°C , leukocytes were obtained, and washed with Pipes A (119mM NaCl, 5mM KCl, 25mM Pipes, 0.03% human serum albumin; pH 7.4) containing 4mM ethylenediaminetetraacetic acid, washed again with Pipes A and finally suspended in an adequate amount of Pipes ACM (Pipes A with 2mM Ca^{2+} and 0.5mM Mg^{2+}).

Histamine release

Leukocytes in 0.2 ml of Pipes ACM were preincubated with 0.2 ml of various concentrations of fenoterol in the absence or presence of theophylline for 15 min at 37°C in 12×75 mm polystyrene tubes. Histamine release reaction was initiated by the addition of 0.1 ml of D.f., anti-IgE, ionophore A23187 or FMLP. Incubation was carried out at 37°C for 45 min. The final cell concentrations of reaction mixture were 1 to $6 \times 10^6/\text{ml}$. Final concentrations of D.f., anti-IgE, ionophore A23187 and FMLP were $0.1 \mu\text{g}/\text{ml}$, 1:1000 (or 1:10000), $0.5 \mu\text{g}/\text{ml}$ and 10^{-4} M, respectively.

When the relationship between the levels of histamine release and the effect of fenoterol was examined, three different concentrations of D.f. were used to obtain a wide range of release.

The tubes were then centrifuged at $400 \times g$ for 15 min at 4°C . Supernatants were stored at 4°C until histamine assay. An aliquot of cells in 0.5 ml of Pipes ACM was lysed with perchloric acid (final concentration : 2%) to evaluate total

cellular histamine. Experiments were performed in duplicate or triplicate.

Histamine assay

Histamine was determined by an automated fluorometric technique (12). Histamine released was expressed as a percentage of total cellular histamine.

RESULTS

Effect of fenoterol on basophil histamine release

The effect of fenoterol on histamine release from human basophils was studied. Leukocytes were preincubated with 10^{-7} to 10^{-3} M of fenoterol for 15 min at 37°C and stimulated with $0.1 \mu\text{g}/\text{ml}$ of D.f. Fenoterol did not have any effect on D.f.-induced histamine release from basophils taken from seven asthmatic patients allergic to mite (Fig. 1A). Similar results were obtained when leukocytes were preincubated with fenoterol for 0, 2, 5 and 30 min, respectively (data not shown). Similarly, fenoterol did not suppress anti-IgE-induced histamine release from basophils taken from four healthy donors (Fig. 1B). Furthermore, fenoterol did not affect the release of histamine induced by FMLP from basophils obtained from an asthmatic patient, a patient with allergic rhinitis, and two healthy donors (Fig. 1C).

The effect of fenoterol on calcium ionophore A23187-induced basophil histamine release using leukocytes from a patient with allergic rhinitis and two healthy donors was then studied.

There was no effect on histamine release at 10^{-11} to 10^{-4} M of fenoterol (Fig. 1D). At 10^{-3} M, a slight inhibition was observed with % inhibition of 11.8 ± 2.4 ($\bar{x} \pm \text{SEM}$) ($P < 0.05$).

No synergism between fenoterol and theophylline in inhibiting IgE-mediated histamine release

Fenoterol did not inhibit the release of histamine by human basophils stimulated with D.f. However, fenoterol could suppress histamine release in the presence of phosphodiesterase inhibitors which are known to inhibit antigen-

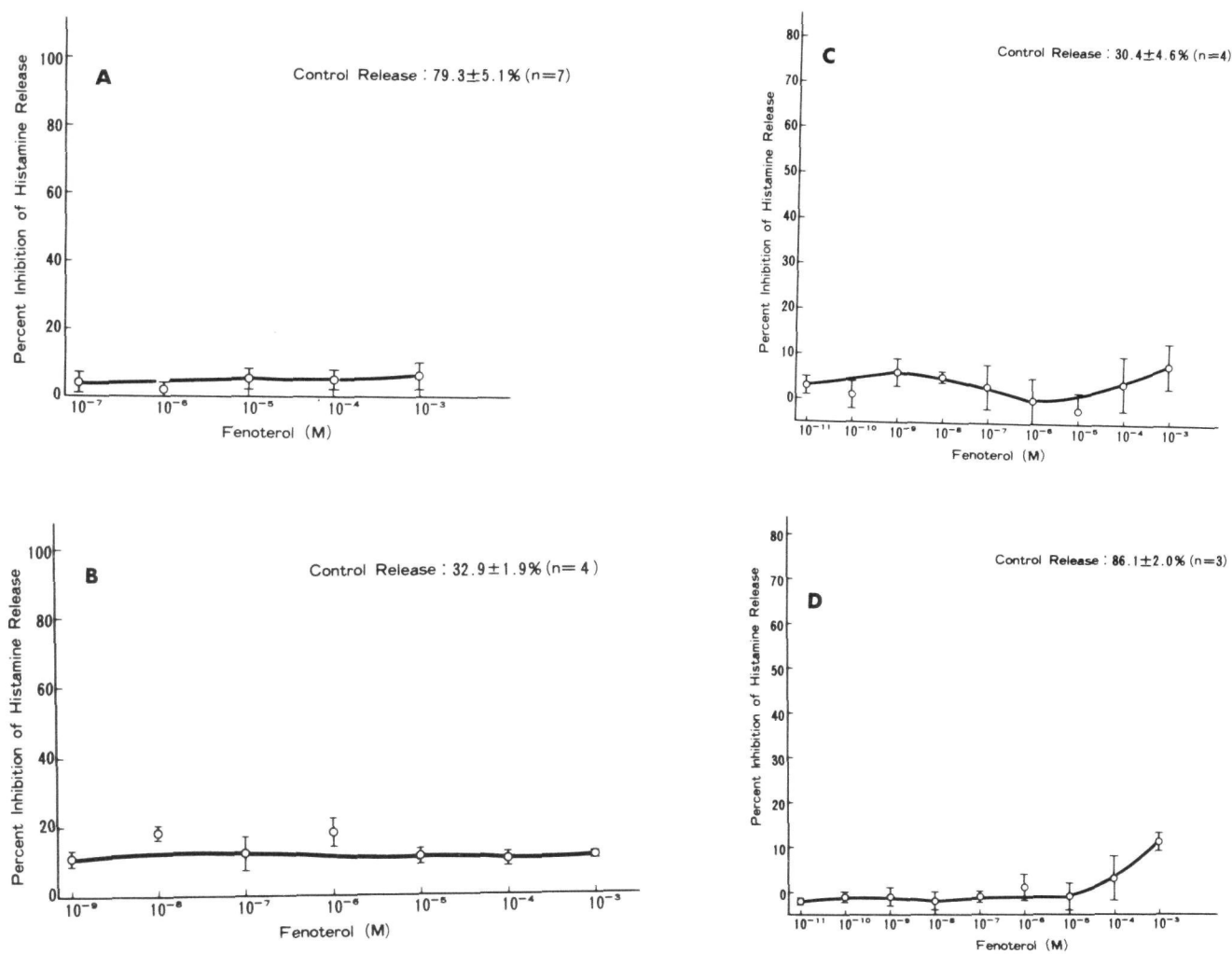


Fig. 1. Effect of fenoterol on basophil histamine release induced by various stimuli. Leukocytes from D.f.-sensitive asthmatic patients, a patient with allergic rhinitis, and healthy donors were preincubated with various concentrations of fenoterol for 15 min at 37°C, and stimulated by various stimuli. D.f. allergen, anti-IgE, FMLP, and the calcium ionophore A23187 were used as stimuli for histamine release in Fig. 1A, B, C, and D, respectively. The numbers in parentheses indicate those of donors.

induced basophil histamine release by increasing cellular levels of cAMP (5). Therefore, the effect of fenoterol plus theophylline on D.f.-induced histamine release was tested.

There was no synergism between fenoterol and theophylline (Fig. 2).

Relationship between effect of fenoterol and levels of histamine release

Fenoterol did not reduce D.f.-induced histamine release. In these experiments, high control release was obtained ($79.3 \pm 5.1\%$). Therefore, we tested the effect of fenoterol on various levels of histamine release induced by D.f.

As shown in Fig. 3, there was no significant inhibition of antigen-induced histamine release by fenoterol at low as well as high levels of release.

DISCUSSION

Antigen-induced IgE-mediated histamine and slow-reacting substance of anaphylaxis (SRS-A) release from human lung fragments is inhibited by the beta-adrenergic agonists (1, 9). IgE-mediated histamine release from dispersed human lung mast cells obtained by enzymatic digestion and purified human lung mast cells by

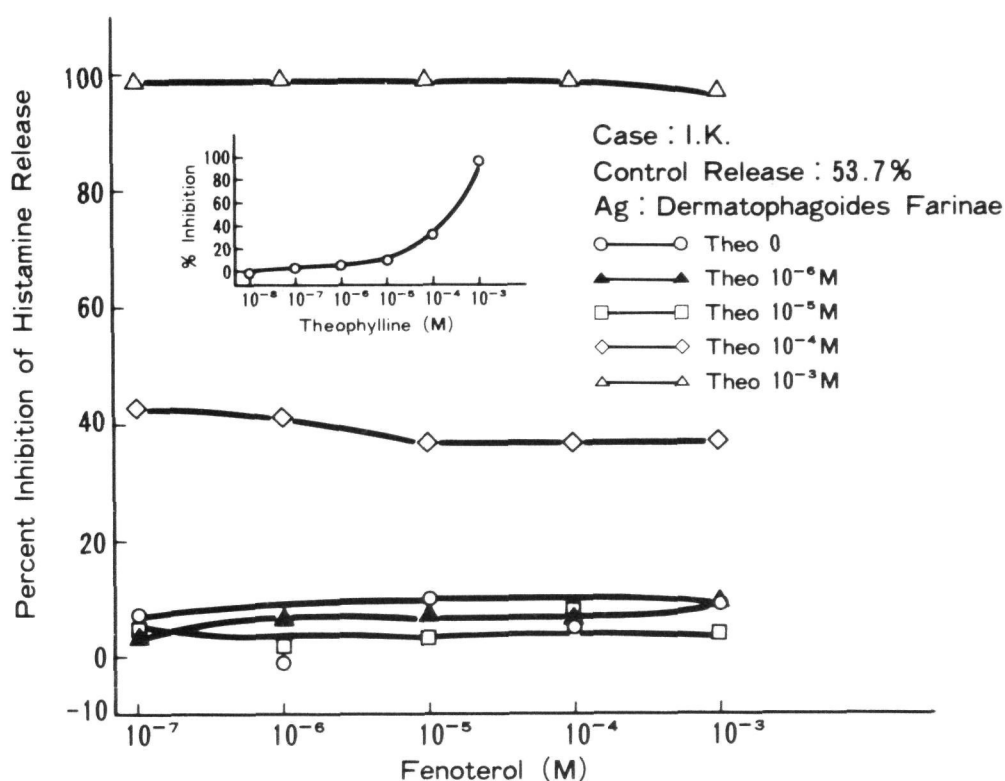


Fig. 2. No synergism between fenoterol and theophylline in inhibiting antigen-induced histamine release. Leukocytes were preincubated with 10^{-7} to 10^{-3} M of fenoterol in the absence or presence of theophylline for 15 min at 37°C . Mite antigen ($0.1 \mu\text{g/ml}$) was then added.

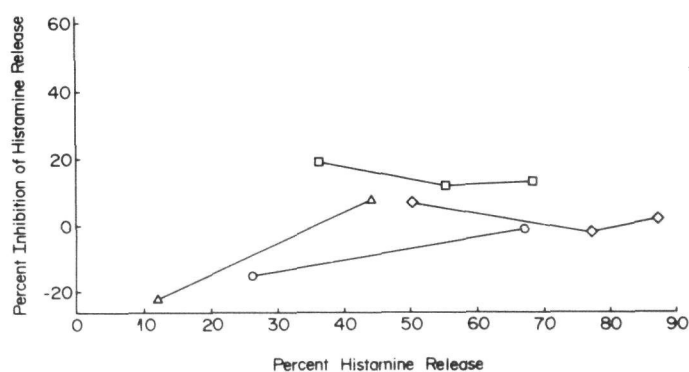


Fig. 3. Relationship between the effect of fenoterol and the levels of histamine release. Leukocytes from allergic patients sensitive to mite were stimulated with various concentrations of D.f to obtain different levels of histamine release.

nergic receptor-adenylate cyclase system in human lung mast cells.

In the present experiments, we demonstrated that antigen and anti-IgE-induced histamine release from human basophils was not inhibited by fenoterol. Furthermore, there was no synergism between fenoterol and theophylline in inhibiting D.f.-induced histamine release. These results clearly indicate that the beta-adrenergic receptor-adenylate cyclase system is not a control mechanism in IgE-mediated basophil histamine release.

It has been reported that human granulocytes and lymphocytes possess beta-adrenergic receptors (15). However, beta-adrenergic receptor-mediated changes in cAMP levels induced by beta-adrenergic agents are different between two cell types; lymphocytes respond much better to beta-adrenergic agents than granulocytes (7). CAMP response to beta-adrenergic agonists might also be poor in human basophils.

The existence of beta-adrenergic receptors in

elutriation and affinity column chromatography is also reduced by fenoterol (10, 11). These observations indicate that IgE-mediated histamine release is modulated by the beta-adre-

rat mast cells has been demonstrated, and there was an elevation in intracellular cAMP in response to fenoterol (8). Nevertheless, suppression of IgE-mediated histamine release by fenoterol was not noted (8).

Further studies have to be done to know which mechanism is involved in the inability of fenoterol to inhibit IgE-mediated basophil histamine release.

Tung et al. reported that cAMP active drugs were more potent inhibitors at low levels of histamine release than at high levels (14). However, we could not obtain similar results. Fenoterol did not inhibit significantly antigen-induced histamine release at low as well as high levels of release.

Fenoterol did not cause an inhibition of histamine release induced by the calcium ionophore A23187 at 10^{-11} to 10^{-4} M. At 10^{-3} M, a slight inhibition was observed. These results are consistent with a previous report (6). Furthermore, fenoterol did not reduce FMLP-induced basophil histamine release. The release of histamine induced by this stimulus has been reported to be inhibited by dibutyryl cAMP (13). The discrepancy suggests that beta-adrenergic agonists do not increase intracellular cAMP levels in human basophils.

In summary, IgE-mediated histamine release from human basophils was not inhibited by fenoterol. This finding indicates that in human basophils, the beta-adrenergic receptor-adenylate cyclase system does not regulate IgE-mediated histamine release.

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REFERENCES

1. Assem, E. S. K. & Schild, H. O.: Inhibition by sympathomimetic amines of histamine release induced by antigen in passively sensitized human lung. *Nature* 224, 1028-1029, 1969.
2. Frey, M. J., Chesrown, S. E., Reed, B. R. et al.: Immunologic release of histamine from dog lung: comparison of in vivo and in vitro responses in the same animal. *J. Allergy Clin. Immunol.* 74, 728-737, 1984.
3. Ishizaka, T., Ishizaka, K., Orange, R. P. & Austen, K. F.: Pharmacologic inhibition of the antigen-induced release of histamine and slow reacting substance of anaphylaxis (SRS-A) from monkey lung tissues mediated by human IgE. *J. Immunol.* 106, 1267-1273, 1970.
4. Koopman, W. J., Orange, R. P. & Austen, K. F.: Immunochemical and biologic properties of rat IgE. III. Modulation of the IgE-mediated release of slow-reacting substance of anaphylaxis by agents influencing the level of cyclic 3',5'-adenosine monophosphate. *J. Immunol.* 105, 1096-1102, 1970.
5. Lichtenstein, L. M. & Margolis, S.: Histamine release in vitro: Inhibition by catecholamine and methylxanthines. *Science* 161, 902-903, 1968.
6. Lichtenstein, L. M.: The mechanism of basophil histamine release induced by antigen and by the calcium ionophore A23187. *J. Immunol.* 114, 1692-1699, 1975.
7. Marone, G., Thomas, L. L. & Lichtenstein, L. M.: The role of agonists that activate adenylate cyclase in the control of cAMP metabolism and enzyme release by human polymorphonuclear leukocytes. *J. Immunol.* 125, 2277-2283, 1980.
8. Marquardt, D. L. & Wasserman, S. I.: Characterization of the rat mast cell beta-adrenergic receptor in resting and stimulated cells by radioligand binding. *J. Immunol.* 129, 2122-2127, 1982.
9. Orange, R. P., Austen, W. G. & Austen, K. F.: Immunological release of histamine and slow-reacting substance of anaphylaxis from human lung. I. Modulation by agents influencing cellular levels of cyclic 3',5'-adenosine monophosphate. *J. Exp. Med.* 134, 136-148, 1971.
10. Peters, S. P., Schulman, E. S., Schleimer, R. P., Macglashan, D. W. Jr., Newball, H. H. & Lichtenstein, L. M.: Dispersed human lung mast cells. *Am. Rev. Respir. Dis.* 126, 1034-1039, 1982.
11. Schulman, E. S., Macglashan, D. W. Jr., Peters, S. P., Schleimer, R. P., Newball, H. H. & Lichtenstein, L. M.: Human lung mast cells: Purification and characterization. *J. Immunol.* 129, 2662-2667, 1982.
12. Siraganian, R. P.: An automated continuous flow system for the extraction and fluorometric analysis of histamine. *Anal. Biochem.* 57, 383-394, 1974.
13. Siraganian, R. P. & Hook, W. A.: Mechanism of histamine release by formyl methionine-containing peptides. *J. Immunol.* 119, 2078-2083, 1977.
14. Tung, R. S. & Lichtenstein, L. M.: Cyclic AMP agonist inhibition increases at low levels of histamine release from human basophils. *J. Pharmacol. Exp. Ther.* 218, 642-646, 1981.
15. Williams, L. T., Snyderman, R. & Lefkowitz, R. J.: Identification of B-adrenergic receptors in human lymphocytes by (-) [^3H] alprenolol binding. *J. Clin. Invest.* 57, 149-155, 1976.

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