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Inhibitory mechanisms of β-adrenoceptor agonists for immunoglobulin E-mediated experimental allergic reactions in rats

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Abstract

Inhibitory mechanisms of isoproterenol and clenbuterol for immunoglobulin E (IgE)-mediated experimental allergic reactions in rats were studied. IgE-mediated passive cutaneous anaphylaxis, histamine-induced cutaneous reaction and serotonin-induced cutaneous reaction were evoked at the same time in the same rats. Isoproterenol administered intravenously immediately before challenge inhibited all these reactions significantly. Clenbuterol administered intravenously 0–3 h before challenge also significantly inhibited the three cutaneous reactions. The inhibition was maximum when the drug was given 1 h before challenge. Passive cutaneous anaphylaxis was always inhibited more potently than histamine-induced cutaneous reaction and serotonin-induced cutaneous reaction by these β-adrenoceptor agonists. Passive peritoneal anaphylaxis was caused by injecting an antigen intravenously. Isoproterenol administered intravenously immediately before challenge inhibited the reaction significantly. Clenbuterol administered intravenously 0–3 h before challenge also significantly inhibited passive peritoneal anaphylaxis, maximally so when given 1 h before challenge. In vitro IgE-dependent histamine release from sensitized peritoneal mast cells or mesenteric mast cells was not affected by isoproterenol and clenbuterol. Mouse monoclonal IgE, a foreign protein, administered intravenously decreased rapidly in the circulation. About 50% of the mouse IgE given disappeared in 20 min. The decrease of mouse IgE was partly but significantly inhibited by the β-adrenoceptor agonists, and the inhibition was abolished by simultaneous treatment with propranolol. These results indicate that direct inhibition of mast cell activation does not contribute to the potent inhibition of in vivo allergic reactions in rats by β-adrenoceptor agonists, and that inhibition of the allergic cutaneous reaction is partially explained by the inhibition of vascular permeability increases caused by mast cell mediators. Penetration of intravenously administered antigen from blood vessels to peripheral tissues to cause mast cell activation might be inhibited by β-adrenoceptor agonists, and this could play some role in inhibiting intravenous antigen-induced allergic reactions in rats. Clenbuterol exhibited its maximum action with some latency in vivo, suggesting that some time-requiring process may be involved in the manifestation of its action.

Keywords: β-Adrenoceptor agonist; Isoproterenol; Clenbuterol; Mast cell; Allergic reaction; Vascular permeability

1. Introduction

β-Adrenoceptor agonists exhibit a potent bronchodilating effect and are used as bronchodilators for the treatment of asthma (Barnes et al., 1984; Tattersfield, 1986). β-Adrenoceptor agonists have a potent relaxing effect on contracted smooth muscles, accompanied by an increase in intracellular cyclic AMP (Barnes, 1986). It is also reported that β-adrenoceptor agonists possess anti-allergic and anti-inflammatory properties. β-Adrenoceptor agonists inhibit immunoglobulin E (IgE)-dependent mediator release from human lung fragments, mast cells and basophils (Orange et al., 1971; Bourne et al., 1972; Butchers et al., 1979; Peters et al., 1982; Hughes et al., 1983), guinea pig and monkey lung fragments (Ishizaka et al., 1971; Undem and Buckner, 1984; Undem et al., 1985), edema formation in mice and rats (Green, 1972; Maling et al., 1974), and vascular permeability increases in rats and hamsters (Svensjö et al., 1977; Ohuchi et al., 1987; Inagaki et al., 1989). In rats, however, β-adrenoceptor agonists do not exhibit potent inhibition of mediator release from mast cells, although in vivo allergic reactions are strongly inhibited by the agents (Barrett-Bee and Lees, 1978; Butchers et al., 1979; Marquardt and Wasserman, 1982; Komoriya et al., 1984; Miyao et al., 1984; Inagaki et al., 1992). Marquardt and Wasserman (1982) demonstrated that histamine release from rat peritoneal mast cells was not inhibited by

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β-adrenoceptor agonists in spite of the increased intracellular cyclic AMP levels. Although it is apparent that the inhibition of vascular permeability increases by β-adrenoceptor agonists contributes to their potent inhibition of some allergic reactions in rats, the inhibitory mechanisms of β-adrenoceptor agonists have not yet been explained completely. Previously we examined the effects of β-adrenoceptor agonists on the allergic reactions in rats, and suggested that some mechanism(s) other than the inhibition of the vascular permeability increase may also contribute to the inhibition of allergic reactions (Inagaki et al., 1992).

In the present study, we further investigated the inhibitory mechanisms of β-adrenoceptor agonists for in vivo allergic reactions in rats. We used for the purpose isoproterenol, a prototype of β-adrenoceptor agonists, and clenbuterol, a long-lasting β2-adrenoceptor selective agonist (Engelhardt, 1976).

2. Materials and methods

2.1. Rats

Male Wistar rats weighing 250–300 g were used throughout. The rats were purchased from Japan SLC (Hamamatsu, Japan). Experiments were undertaken following guidelines for the care and use of the experimental animals from the Japanese Association for Laboratory Animal Science (1987).

2.2. Antigens, antiserum and monoclonal IgE

Dinitrophenol-conjugated *Ascaris suum* extract (Strejan and Campbell, 1967) and bovine serum albumin were prepared according to the method described by Eisen et al. (1953) and used as antigens.

Rat antiserum against dinitrophenol-conjugated *Ascaris suum* extract was prepared according to the method described by Tada and Okumura (1971) with a slight modification. The IgE titer of the antiserum preparation estimated by homologous passive cutaneous anaphylaxis was 1:512. Rat anti-dinitrophenol monoclonal IgE was prepared as reported previously (Inagaki et al., 1994). The IgE titer of the preparation estimated by passive cutaneous anaphylaxis was 1:2000 or greater. Mouse anti-dinitrophenol monoclonal IgE was prepared similarly (Sakurai et al., 1994). The IgE concentration of the mouse IgE preparation estimated by enzyme-linked immunosorbent assay (ELISA, described below) was 1415 ng/ml.

2.3. Drugs

Isoproterenol (hydrochloride) and propranolol (hydrochloride) were purchased from Sigma (St. Louis, MO, USA). Clenbuterol (hydrochloride) was a generous gift from Teijin (Tokyo, Japan). Drugs were dissolved in saline and administered intravenously in in vivo experiments. In in vitro experiment drugs were dissolved in the medium used in the experiments.

2.4. Cutaneous reactions

IgE-mediated homologous passive cutaneous anaphylaxis, histamine-induced cutaneous reaction and serotonin-induced cutaneous reaction were evoked at the same time in the same rats as reported previously (Koda et al., 1990). In brief, rats were passively sensitized by an intradermal injection of 100 μl of 30-fold diluted rat antiserum against dinitrophenol-conjugated *Ascaris suum* extract. Two days later, 10^{-5} g/ml of histamine (dihydrochloride, Nacalai Tesque, Kyoto, Japan) and 5×10^{-7} g/ml of serotonin (creatinin sulfate, Merck, Darmstadt, Germany), in a volume of 100 μl, was injected intradermally. Immediately after the injection of vascular permeability increasing factors, the rats received an intravenous injection of 1 mg of dinitrophenol-conjugated bovine serum albumin and 5 mg of Evans blue dye. 30 min later, the rats were killed and the reaction sites were excised. Extravasated dye in the excised skin sites was extracted and evaluated colorimetrically according to the method described by Katayama et al. (1978). The amount of extravasated dye in control experiments under the present experimental conditions was usually as follows: passive cutaneous anaphylaxis, 20–30 μg/site; histamine-induced cutaneous reaction, about 20 μg/site, serotonin-induced cutaneous reaction, about 20 μg/site.

2.5. Passive peritoneal anaphylaxis

Histamine release in vivo in the peritoneal cavity of rats was obtained according to the method described previously (Orange et al., 1968, 1970; Ross et al., 1976) with some modifications. Rats were passively sensitized by an intraperitoneal injection of 2 ml of 20-fold diluted rat antiserum against dinitrophenol-conjugated *Ascaris suum* extract. Two days later, histamine release was evoked by injecting 1 mg of dinitrophenol-conjugated *Ascaris suum* extract intravenously. Exactly 2 min later, the rats were killed and bled carefully. Two minutes after killing, 10 ml of Tyrode solution (137 mM NaCl, 2.7 mM KCl, 0.41 mM NaH_2PO_4, 1.6 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% glucose, 10 mM HEPES, 0.05% gelatin, pH 7.4) was injected into the peritoneal cavity and massaged gently. The peritoneal washings were recovered 2 min after injection of Tyrode solution. After centrifugation at $350 \times g$ for 10 min, the supernatants were separated off. Histamine in the supernatant was measured fluorometrically (May et al., 1970) by a post-column derivatization method on an automated histamine analyzing system (Tosoh, Tokyo) (Yamatodani et al., 1985; Inagaki et al., 1992). Under the present experimental conditions, about 20 ng/ml of histamine was detected spontaneously. The concentration of specifically

released histamine in the peritoneal washings was 160–190 ng/ml.

2.6. Histamine release from peritoneal mast cells

In vitro histamine release from peritoneal mast cells was examined as reported previously (Inagaki et al., 1994). Briefly, the rats were passively sensitized by an intraperitoneal injection of 2 ml of 200-fold diluted rat monoclonal IgE preparation. Two day later, peritoneal exudate cells were recovered using Tyrode solution containing 5 u/ml of heparin. Cells were washed twice with Tyrode solution, and suspended in Tyrode solution at a concentration of 10^5 mast cells/ml. Histamine release was initiated by adding dinitrophenol-conjugated bovine serum albumin at a final concentration of 1 μg/ml. After incubation with antigen at 37°C for 15 min, the reaction was terminated and the supernatant was separated by centrifugation at $350 \times g$ for 10 min. To assess the amount of total histamine, cell-associated histamine was extracted in the presence of 1.2% $HClO_4$. Released histamine in the supernatant was measured fluorometrically as mentioned above. The mast cell histamine content was 30–40 μg/10^6 cells, and the spontaneous release of histamine was 3% of total histamine or lower. Peritoneal mast cells released about 30% of their total histamine upon challenge with 1 μg/ml dinitrophenol-conjugated bovine serum albumin. Duplicate experiments with the same or a similar design were repeated at least twice, and a representative experiment is shown.

2.7. Histamine release from mesenteric mast cells

The mesenterium of rats was excised and cut into pieces in Tyrode solution. Over 10 specimens could be prepared from one rat. The mesenterium specimens were incubated in 20-fold diluted rat monoclonal IgE preparation at 4°C overnight. The mesenterium specimens were rinsed with, and then incubated in Tyrode solution at 37°C for 1 h to remove unbound IgE. A piece of mesenterium specimen placed in a tube with Tyrode solution was stimulated with dinitrophenol-conjugated bovine serum albumin at a final concentration of 1 μg/ml. After incubation with antigen at 37°C for 15 min, the reaction was terminated. After separation of the mesenterium, the supernatant was centrifuged at $350 \times g$ for 10 min. The residual histamine in the mesenterium was extracted in the presence of 1.2% $HClO_4$ at room temperature for 30 min. Under these conditions over 96% of tissue-associated histamine was extracted. Histamine was measured fluorometrically as mentioned above. Total histamine was calculated from the amounts of released and unreleased histamine. Spontaneous release was 2% of total histamine or less, and mesenteric mast cells released about 50% of their total histamine under the present experimental conditions.

2.8. Examination of mouse monoclonal IgE given intravenously

Under ether anesthesia, rats received an intravenous injection of 0.5 ml of mouse monoclonal IgE preparation into their tail vein. Exactly 2 and 20 min later, blood samples were obtained from the orbital sinus and sera were separated.

Mouse IgE in the sera was determined by enzyme-linked immunosorbent assay (ELISA). In brief, a microtiter plate (Immunoplate, Nunc) was coated with monoclonal anti-mouse IgE antibody (heavy chain-specific, rat IgG1, Serotec), and samples and standard IgE (mouse monoclonal anti-dinitrophenol IgE, Sigma) at appropriate dilutions were added. After incubation, the plate was washed, and biotin-labeled dinitrophenol-conjugated bovine serum albumin was added and incubated. Biotin-labeled dinitrophenol-conjugated bovine serum albumin was prepared by conjugating biotin (ImmunoPure NHS-LC-Biotin, Pierce) to dinitrophenol-conjugated bovine serum albumin. After washing, peroxidase-labeled streptavidin (Dakopatts) was added and reacted to the biotin residue trapped on the plate. Finally, the enzyme reaction was performed by adding substrate solution and the product was evaluated colorimetrically at 450 nm by a plate reader (Titertec Multiscan MCC/340, Flow Laboratory). The concentration of mouse IgE at 2 min after its injection was about 50 ng/ml.

2.9. Statistical analysis

Statistical significance of the data expressed as the mean value and its standard error was performed using Duncan's multiple range test or Student's *t*-test. When uniform variance of data was identified by Bartlett's analysis ($P < 0.05$), one-way analysis of variance was used to test for statistical differences. Significant differences ($P < 0.05$) were identified, after which the data were further analyzed by Duncan's multiple range test for significant differences between individual pairs of means. In experiments containing 2 experimental groups, the Student's *t*-test was employed after the variance of the data had been examined.

3. Results

3.1. Effects of β-adrenoceptor agonists on cutaneous reactions

Passive cutaneous anaphylaxis, histamine-induced cutaneous reaction and serotonin-induced cutaneous reaction were evoked at the same time in the same rats, and the effects of isoproterenol and clenbuterol on the cutaneous reactions were examined. The drugs were given intravenously 0–12 h before challenge.

As shown in Fig. 1A, isoproterenol at a dose of 10 μg/kg administered intravenously immediately before challenge significantly inhibited the three cutaneous reactions. Histamine- and serotonin-induced cutaneous reactions were inhibited by about 70% although passive cutaneous anaphylaxis was inhibited by over 90%. Isoproterenol when given immediately before challenge inhibited the three cutaneous reactions in a dose-dependent manner (Fig. 1B). The inhibition of passive cutaneous anaphylaxis was more potent than those of histamine- and serotonin-induced cutaneous reactions.

Clenbuterol at a dose of 10 μg/kg administered intravenously 0–3 h before challenge significantly inhibited the three cutaneous reactions (Fig. 1C). Passive cutaneous anaphylaxis was also significantly inhibited when the drug was given 6 h before, although histamine- and serotonin-induced cutaneous reactions were only slightly inhibited. The inhibition was maximum when the drug was given 1 h before challenge. As shown in Fig. 1D, clenbuterol given 1 h before challenge inhibited the three cutaneous reactions in a dose-dependent manner. Passive cutaneous anaphylaxis was always inhibited more potently than the other cutaneous reactions.

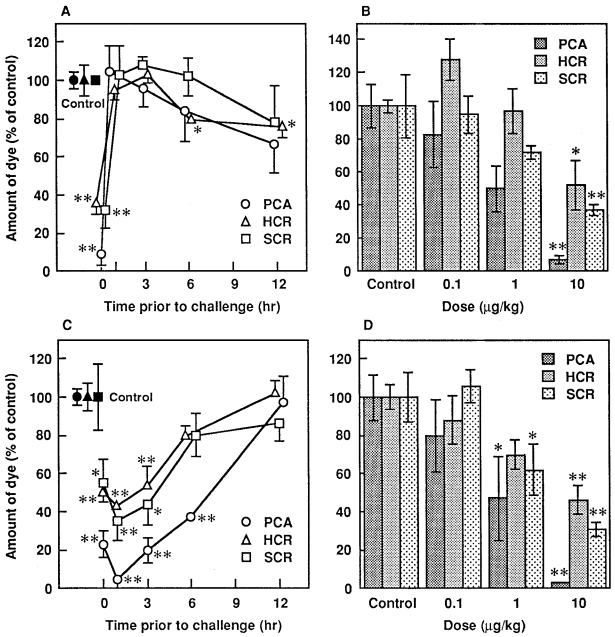

Fig. 1. Effects of isoproterenol and clenbuterol on passive cutaneous anaphylaxis (PCA), histamine-induced cutaneous reaction (HCR) and serotonin-induced cutaneous reaction (SCR) elicited at the same time in the same rats. (A) Isoproterenol in a dose of 10 μg/kg was administered intravenously 0–12 h prior to elicitation of reactions. (B) Isoproterenol in doses of 0.1–10 μg/kg was administered intravenously just before elicitation of reactions. (C) Clenbuterol in a dose of 10 μg/kg was administered intravenously 0–12 h prior to elicitation of reactions. (D) Clenbuterol in doses of 0.1–10 μg/kg was administered intravenously 1 h prior to elicitation of reactions. Each value represents the mean ± S.E.M. for 4 rats. * $P < 0.05$, ** $P < 0.01$, by Duncan's multiple range test.

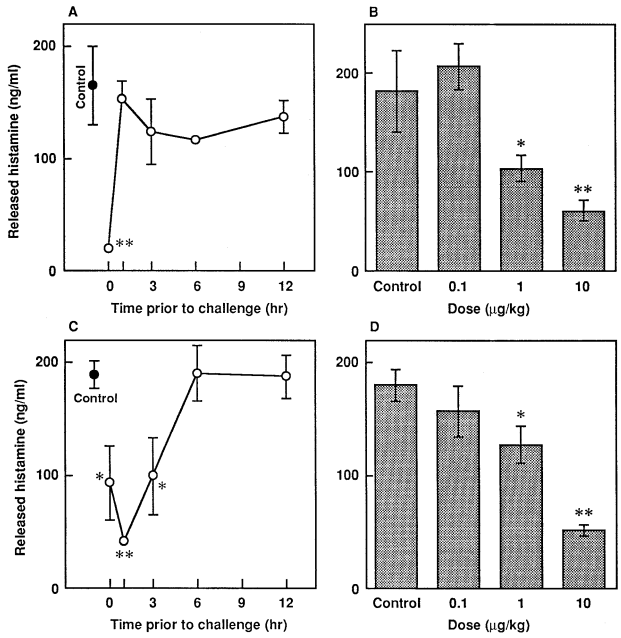

Fig. 2. Effects of isoproterenol and clenbuterol on antigen-induced histamine release in the rat peritoneal cavity. (A) Isoproterenol in a dose of 10 μg/kg was administered intravenously 0–12 h prior to elicitation of reaction. (B) Isoproterenol in doses of 0.1–10 μg/kg was administered intravenously just before elicitation of reaction. (C) Clenbuterol in a dose of 10 μg/kg was administered intravenously 0–12 h prior to elicitation of reaction. (D) Clenbuterol in doses of 0.1–10 μg/kg was administered intravenously 1 h prior to elicitation of reactions. Each value represents the mean ± S.E.M. for 4 rats. * $P < 0.05$, ** $P < 0.01$, by Duncan's multiple range test.

3.2. Effects of β-adrenoceptor agonists on histamine release in vivo

In vivo histamine release was examined by means of passive peritoneal anaphylaxis. The β-adrenoceptor agonists were given intravenously 0–12 h before challenge.

As shown in Fig. 2A, 10 μg/kg of isoproterenol administered intravenously immediately before challenge inhibited histamine release significantly. The inhibition by isoproterenol given immediately before challenge was dose-dependent (Fig. 2B).

Clenbuterol at a dose of 10 μg/kg administered intravenously 0–3 h before challenge also significantly inhibited the histamine release (Fig. 2C). The inhibition was maximum when the drug was given 1 h before challenge, as in the case of cutaneous reactions. Clenbuterol given 1 h before challenge inhibited the histamine release in a dose-dependent manner (Fig. 2D).

3.3. Effects of β-adrenoceptor agonists on histamine release in vitro

IgE-dependent in vitro histamine release was examined using peritoneal mast cells and mesenteric mast cells.

Results for peritoneal mast cells are shown in Fig. 3A and B. Mast cells were incubated in the presence of isoproterenol or clenbuterol at concentrations of 10^{-10}–10^{-5} M for 5 min before antigen stimulation. Isoproterenol failed to affect the histamine release from mast cells (Fig. 3A). Clenbuterol also failed to affect the histamine release significantly: histamine release was reduced by only about 20% when mast cells were incubated in the presence of 10^{-5} M clenbuterol (Fig. 3B).

Results for mesenteric mast cells are shown in Fig. 3C and D. Mesenteric mast cells were incubated in the presence of 10^{-10}–10^{-5} M of isoproterenol or clenbuterol for 5 min and stimulated with antigen. Neither isoproterenol (Fig. 3C) nor clenbuterol (Fig. 3D) affected histamine release.

3.4. Effects of β-adrenoceptor agonists on the fate of mouse IgE

To examine the effects of β-adrenoceptor agonists on the fate of intravenously administered antigen, a foreign protein, in the rat circulation, the concentration of mouse IgE given intravenously was measured. Blood samples were obtained 2 and 20 min after the injection of mouse monoclonal IgE preparation, and the results were expressed as the concentration at 20 min as a percentage of that at 2 min. As shown in Fig. 4, mouse IgE administered intravenously decreased rapidly in the circulation. About 50% of the mouse IgE given disappeared in 20 min. Isoproterenol and clenbuterol at a dose of 10 μg/kg were administered immediately before and 1 h before the injection of mouse IgE, respectively. The decrease of mouse IgE was partly but significantly inhibited by both isoproterenol and clenbuterol (Fig. 4A and C). The inhibition by the β-adrenoceptor agonists was counteracted by the simultaneous treatment with 1 mg/kg of propranolol 10 min before (Fig. 4B and D).

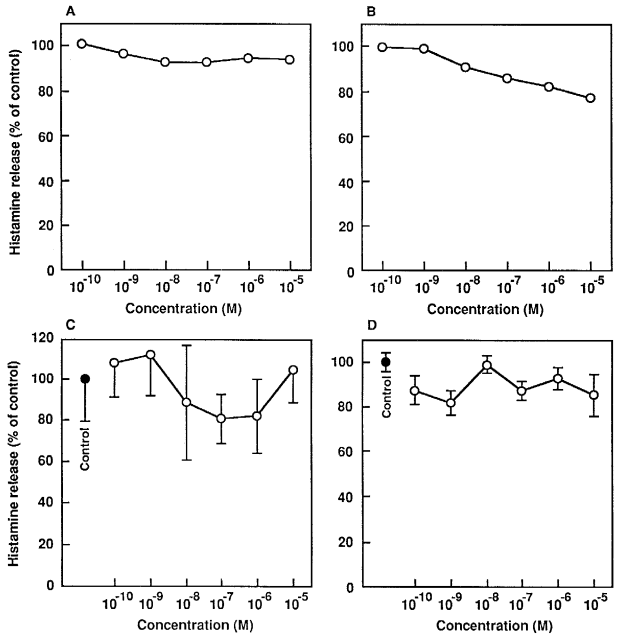

Fig. 3. Effects of isoproterenol and clenbuterol on antigen-induced histamine release from rat peritoneal mast cells and mesenteric mast cells. Peritoneal mast cells were incubated for 5 min with 10^{-10}–10^{-5} M of isoproterenol (A) or clenbuterol (B) before challenge. Each value represents the mean of duplicate determinations in one representative experiment. Mesenterium was also incubated for 5 min with 10^{-10}–10^{-5} M of isoproterenol (C) or clenbuterol (D) before challenge. Each value represents the mean ± S.E.M. for 4 rats.

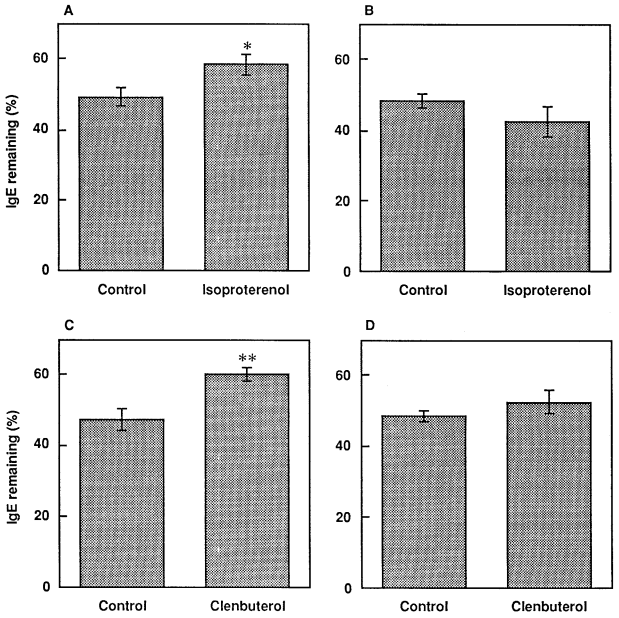

Fig. 4. Effects of isoproterenol (A and B) and clenbuterol (C and D) on the disappearance of mouse monoclonal IgE from the rat circulation in the absence (A and C) or presence (B and D) of propranolol treatment. Mouse monoclonal IgE was injected into the tail vein of rats and blood samples were obtained from the orbital sinus 2 and 20 min later. Mouse IgE in the serum was measured by ELISA. Isoproterenol at a dose of 10 μg/kg was administered intravenously immediately before the IgE injection, and the same dose of clenbuterol was given intravenously 1 h prior to the IgE injection. Propranolol at a dose of 1 mg/kg was given intravenously 10 min before the β-adrenoceptor agonist injection. The concentration of mouse IgE remaining in the serum at 20 min was expressed as a percentage of that at 2 min. Each value represents the mean ± S.E.M. for 4 or 5 rats. $^{*}P < 0.05$, $^{**}P < 0.01$, by Student's *t*-test.

4. Discussion

In the present study, we have confirmed the previous reports that β-adrenoceptor agonists inhibit rat allergic reactions in spite of their lesser effectiveness for inhibition of mast cell histamine release (Barrett-Bee and Lees, 1978; Butchers et al., 1979; Marquardt and Wasserman, 1982;

Komoriya et al., 1984; Miyao et al., 1984; Inagaki et al., 1992), and indicated that β-adrenoceptor agonists regulate the distribution of foreign protein antigen given intravenously from blood vessels to peripheral tissues. The inhibition of antigen distribution could play some role in the inhibition of experimental allergic reactions in rats.

Isoproterenol and clenbuterol at a dose of 10 μg/kg almost completely inhibited the IgE-dependent allergic cutaneous reaction, passive cutaneous anaphylaxis. On the contrary, the vascular permeability increase caused by histamine and serotonin tested simultaneously in the same rats was not inhibited in parallel with that of passive cutaneous anaphylaxis. Histamine and serotonin are major vascular permeability-increasing factors stored in rodent mast cells (Harvima and Schwartz, 1993). Passive cutaneous anaphylaxis was consistently inhibited more potently than histamine- and serotonin-induced cutaneous reactions, although the inhibition of histamine- and serotonin-induced cutaneous reactions was almost comparable. Furthermore, as indicated in Fig. 1C (at 6 h), clenbuterol inhibited passive cutaneous anaphylaxis by over 60% without significant inhibition of the other cutaneous reactions in the same rats. It is clear, therefore, that there are mechanism(s) other than inhibition of the vascular permeability increase also involved in the inhibition of passive cutaneous anaphylaxis as suggested before (Inagaki et al., 1992).

In vivo histamine release was examined by means of passive peritoneal anaphylaxis (Orange et al., 1968, 1970; Ross et al., 1976). In the present study, the reaction was evoked by injecting antigen intravenously, as with passive cutaneous anaphylaxis. Isoproterenol and clenbuterol inhibited the passive peritoneal anaphylaxis significantly, although the inhibition was less potent in the case of passive cutaneous anaphylaxis. The difference may be explained by the degree of participation of the vascular permeability increase in these reactions. The histamine release reduction by the β-adrenoceptor agonists is reflected by reduced mast cell activation.

Contrary to the profound inhibition of in vivo IgE-dependent allergic reactions by β-adrenoceptor agonists, the inhibition of IgE-dependent histamine release from peritoneal mast cells and mesenteric mast cells was only limited. Mesenteric mast cells and peritoneal mast cells are considered to be the major source of histamine in passive peritoneal anaphylaxis, and both peritoneal mast cells and skin mast cells are of the connective tissue type. It is apparent, therefore, that direct inhibition of mast cell activation could not contribute to the inhibition of passive cutaneous anaphylaxis and passive peritoneal anaphylaxis in rats by β-adrenoceptor agonists. However, we need to confirm the effect of β-adrenoceptor agonists on skin mast cells because the character of skin mast cells may not be identical to that of peritoneal mast cells (Barrett et al., 1985).

The results for histamine release both in vivo and in vitro strongly suggest that β-adrenoceptor agonists regulate the distribution of foreign protein antigen, given intravenously, from blood vessels to peripheral tissues. Actually, when passive peritoneal anaphylaxis is elicited by injecting antigen intraperitoneally, the inhibition of the passive peritoneal anaphylaxis by β-adrenoceptor agonists becomes inconsistent (Inagaki et al., 1992). Furthermore, in the present study, we demonstrated that isoproterenol and clenbuterol inhibited the rapid reduction of intravenously given foreign protein concentration in the circulation. An agent given intravenously usually distributes to peripheral tissues quickly, so its concentration in the blood declines quickly to reach an equilibrium. Similarly, intravenously administered antigen distributes to peripheral tissues and causes mast cell activation. Isoproterenol and clenbuterol are considered to inhibit this process, and the action is β-adrenoceptor-mediated. Inhibition of antigen distribution, especially at the initial step after antigen injection, might contribute significantly to the inhibition of experimental allergic reactions by β-adrenoceptor agonists.

In the present study, we employed mouse monoclonal IgE as a model foreign protein, and could examine its concentration in the rat blood using ELISA. In the experimental allergic reactions, antigen molecules have an affinity to mast cell-bound IgE. Similarly, mouse IgE has an affinity to the rat mast cells through their FcεRI. We are now planning to examine the amount of mouse IgE distributed to mast cells.

Isoproterenol is a prototype of β-adrenoceptor agonists, and clenbuterol is a long-lasting agent selective to β2-adrenoceptors (Engelhardt, 1976; Komoriya et al., 1984). Both drugs inhibited the rat allergic reactions significantly and comparably, indicating that the inhibition was mediated through β2-adrenoceptors. The inhibition by isoproterenol was only observed when the drug was administered intravenously immediately before antigen challenge. On the contrary, clenbuterol exhibited its inhibitory effect when given 0–3 h before antigen challenge. In the case of passive cutaneous anaphylaxis, administration of clenbuterol 6 h before challenge also produced potent inhibition. It is interesting to note that the maximum inhibition by clenbuterol was observed when it was given 1 h before challenge even with intravenous administration. It is suggested therefore that some time-requiring process may be involved in exhibiting its action.

The present results indicate both that direct inhibition of mast cell activation does not contribute to the potent inhibition of in vivo allergic reactions in rats by β-adrenoceptor agonists, and that the inhibition of allergic cutaneous reaction is partially explainable by the inhibition of the vascular permeability increase caused by mast cell mediators. Penetration of intravenously administered antigen from blood vessels to peripheral tissues to cause mast cell activation might also be inhibited by β-adrenoceptor agonists, and this could play some role in inhibiting intravenous antigen-induced allergic reactions in rats.

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