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Abstract

Passive cutaneous anaphylaxis (PCA) was produced in the rat with mouse IgE-rich antiserum. The effect of drugs on the PCA-induced skin histamine decrease and leakage of protein-bound dye was studied. Salbutamol (0.5 mg/kg i.v. or 1.0 mg/kg s.c.) and cromoglycate (10 mg/kg i.v.) significantly inhibited the skin histamine decrease. A combination of salbutamol (0.5 mg/kg i.v. or 1.0 mg/kg s.c.) and aminophylline (25 mg/kg i.v. or 75 mg/kg s.c.) had an additive or greater than additive effect on the histamine decrease. Salbutamol (1.0 mg/kg s.c.) inhibited the dye leakage markedly, and aminophylline (75 mg/kg s.c.) slightly. These results indicate that the decrease in the skin histamine content is useful as an index of the in vivo inhibitory effect of antiallergic drugs on the antigen-induced histamine release.

KEYWORDS: passive cutaneous anaphylaxis(PCA), antiallergic drugs, histamine release, dye leakage

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INHIBITION BY DRUGS OF PASSIVE CUTANEOUS ANAPHYLAXIS-INDUCED SKIN HISTAMINE DECREASE

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Abstract. Passive cutaneous anaphylaxis (PCA) was produced in the rat with mouse IgE-rich antiserum. The effect of drugs on the PCA-induced skin histamine decrease and leakage of protein-bound dye was studied. Salbutamol (0.5 mg/kg i.v. or 1.0 mg/kg s.c.) and cromoglycate (10 mg/kg i.v.) significantly inhibited the skin histamine decrease. A combination of salbutamol (0.5 mg/kg i.v. or 1.0 mg/kg s.c.) and aminophylline (25 mg/kg i.v. or 75 mg/kg s.c.) had an additive or greater than additive effect on the histamine decrease. Salbutamol (1.0 mg/kg s.c.) inhibited the dye leakage markedly, and aminophylline (75 mg/kg s.c.) slightly. These results indicate that the decrease in the skin histamine content is useful as an index of the *in vivo* inhibitory effect of antiallergic drugs on the antigen-induced histamine release.

Key words : passive cutaneous anaphylaxis (PCA), antiallergic drugs, histamine release, dye leakage.

Passive cutaneous anaphylaxis (PCA) has been widely used for testing the titer of antisera as well as for screening anti-allergic drugs. For the estimation of the intensity of PCA, a dye such as Evans blue which is easily bound to plasma proteins is injected i.v. simultaneously with the antigen, and the amount of the dye accumulated in the antibody-injected site is determined. The leakage of protein-bound dye is the result of a vascular permeability increase induced by histamine and other vaso-active substances locally released or formed. It does not necessarily follow that an inhibition by drugs of the dye leakage in the PCA site indicates a suppression of the release or formation of the chemical mediators of PCA.

It is well known that histamine and serotonin released from mast cells in the local cutaneous tissue play important roles in IgE-mediated PCA in the rat (1). A long time is required for the recovery of the skin histamine content after its depletion by histamine releasers such as compound 48/80 (2). Consequently, it seems likely that the extent of the histamine release during the course of PCA can be known by determining, at an appropriate time after the induction of PCA, the amount of histamine remaining in the skin of the PCA site and comparing it with the skin histamine content in a suitable control site.

It has been suggested that β-adrenergic agonists and methylxanthines inhibit

the anaphylactic histamine release from mast cells by increasing the cellular cyclic AMP content (3). Since such a view is based mainly on the results of *in vitro* experiments, *in vivo* tests of the effect of these drugs on the anaphylactic histamine release from mast cells are required to elucidate the mode of their *in vivo* action.

In the present study, a marked decrease in the skin histamine content in the rat was indeed observed in the site of heterologous PCA induced by mouse antiserum containing a high titer of IgE antibodies. Thus, taking the decrease in skin histamine levels in the PCA site as an indicator, the effect of salbutamol, a β_2-agonist, and aminophylline, a methylxanthine, on the *in vivo* anaphylactic histamine release in the skin was estimated.

MATERIALS AND METHODS

Animals. Male 11-18 week-old Sprague-Dawley rats weighing 350-470 g (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) and male 8-12 week-old BALB/c mice (Shizuoka Laboratory Animal Center and Mouse Colony of Okayama University Medical School) were used.

Chemicals and drugs. Salbutamol hemisulfate was obtained from Sankyo Co. (Tokyo, Japan); aminophylline from Eisai Co. (Tokyo); disodium cromoglycate from Fujisawa Pharmaceutical Co. (Osaka, Japan); ovalbumin (5 × crystalline, OA) from ICN Pharmaceuticals (Cleveland, O) and Evans blue from E. Merck (Darmstadt, Germany).

Preparation of antisera. Mouse antisera containing high titers of IgE antibodies were prepared according to the method described by Levine and his colleagues (4, 5). Mice were immunized by injecting i.p. 0.2 ml of 0.9 % NaCl containing 1 μg OA and 1 mg Al $(OH)_3$ gel, twice at an interval of 4 weeks. The animals were bled from the carotid artery 7 days after a booster injection. The blood collected from 20-40 mice was pooled, diluted with two volumes of Hanks' balanced salt solution (NaCl, 137 mM; KCl, 5.36 mM; $MgSO_4$, 0.811 mM; Na_2HPO_4, 0.337 mM; KH_2PO_4, 0.440 mM; $NaHCO_3$, 4.17 mM; $CaCl_2$ 1.26 mM; $MgCl_2$, 0.492 mM; glucose, 5.55 mM) and centrifuged at 650 × *g* for 10 min at 4 ℃. The resulting fivefold dilution of antiserum was stored at -20 ℃.

PCA. Rats were fixed on a board in a prone position under light ether anesthesia. A fivefold dilution of the mouse anti-OA serum (0.05 ml) was intradermally injected in the left dorsal region at the level of the superior iliac spine. The dorsal skin had been shaved by an electric clipper one day before. A 27-gauge needle was used for the injection, and the same volume of 0.9 % NaCl was injected into the opposite side as a control. OA (10 mg/kg) dissolved in 0.9 % NaCl (2 mg/ml) was injected i.v. under light ether anesthesia 72 h after the injection of antiserum.

The animals were sacrificed 48 h after the antigen injection by severing the carotid artery. The dorsal and laternal skin was removed. The excised skin was fixed on a board with the hairy side down under appropriate tension applied as evenly as possible. The PCA and control sites were excised with a steel punch of 12 or 15 mm in diameter. When the 12 mm steel punch was used, the weight of skin samples of the control and PCA sites was 144.4 ± 5.2 mg (mean ± S.E.M., n = 6) and 165.5 ± 14.1 mg (n = 6), respectively. When the diameter of the punch was 15 mm, the control and PCA skin samples weighed 292.8 ± 12.5 mg (n = 6) and 315.0 ± 18.4 mg (n = 6), respectively.

Salbutamol was injected i.v. immediately (10-15 sec) or s.c. 30 min before the antigen injection at the doses of 0.5 and 1.0 mg/kg, respectively. Aminophylline was injected i.v. im-

mediately or s.c. 30 min before the antigen at the doses of 25 and 75 mg/kg, respectively. Cromoglycate was injected i.v. immediately before the antigen at the dose of 10 mg/kg. The doses of salbutamol and cromoglycate are expressed as the respective salts. All drugs were dissolved in 0.9 % NaCl, and the volume of the injection was 2.0 ml/kg.

Determination of histamine. Each skin sample was cut into small pieces. One milliliter of 0.9 % NaCl was added per 100 mg of tissue. The mixture was boiled for 5 min and cooled. After the addition of an equal volume of 0.8 N perchloric acid, the mixture was homogenized. The histamine content of each sample was fluorometrically determined by the method of Shore *et al.* (6). After the fluorophore formation, 2 M citric acid instead of 3 N HCl was used as the acidifying agent according to Anton and Sayre (7).

The percent decrease induced by PCA in the skin histamine content was calculated as follows: % decrease = [(the skin histamine content in the control site) - (the skin histamine content in the PCA site)] × 100/(the skin histamine content in the control site).

Estimation of dye leakage. Evans blue (100 mg/kg) was injected i.v. together with OA. The rats were sacrificed 30 min later and the skin samples of the PCA and control sites were excised as described above using a 15 mm steel punch. The dye accumulated in each sample was extracted and determined by the method of Katayama *et al.* (8). Briefly, each chopped skin sample was suspended in 1 ml of 1 N KOH and incubated overnight at 37 ℃. Evans blue was subsequently extracted with 9 ml of a 0.6 N H_3PO_4-acetone mixture (5 : 13, v/v), and the absorbance at 620 nm of the extract was measured. When the absorbance exceeded 2.0, the extract was diluted with an aqueous acetone solution (H_2O : acetone = 5 : 13, v/v). The Evans blue content (μg) of each sample was calculated from the standard curve.

RESULTS

Time course of the decrease in the skin histamine content in the PCA site. The histamine content in the control site was 2.65 ± 0.05 μg (base)/sample (mean ± S.E. M., n = 4), when the skin samples were obtained using the 12-mm steel punch. After the antigen injection, the skin histamine levels in the PCA site rapidly decreased as compared with the levels in the control site, reaching the lowest value within 24 h. The histamine levels remained low for more than 72 h.

We reasoned that the presence of a marked edema in the PCA site would be undesirable for the accurate estimation of the decrease in the histamine content of a given skin area. Therefore, we determined the histamine content 48 h after the challenging injection, when the edema in the PCA site had largely disappeared (see Methods).

Effect of drugs on the PCA-induced decrease in the skin histamine content : experiments with the 12-mm steel punch. In the non-treated control group, the histamine content of the skin samples obtained with the 12-mm steel punch from the PCA site 48 h after the challenging injection was markedly lower than the histamine content of the skin samples obtained from the saline-injected control site.

Salbutamol (0.5 mg/kg) injected i.v. alone immediately before the antigen injection had no significant effect on the PCA-induced decrease in the skin histamine content (Table 1). Aminophylline (25 mg/kg) injected in the same manner had no significant effect either. However, when aminophylline (25 mg/kg) and

TABLE 1. EFFECT OF SALBUTAMOL, AMINOPHYLLINE AND CROMOGLYCATE ON THE PCA-INDUCED DECREASE IN THE SKIN HISTAMINE CONTENT AS DETERMINED BY EXCISING THE SKIN SAMPLES WITH A STEEL PUNCH OF 12 MM IN DIAMETER

Drug[a] (mg/kg)	No. of rats	% Decrease in the histamine content[b]	% Inhibition
Control[c]	10	76.2±2.4	—
Salbutamol (0.5)	6	59.9±7.1	21.4
Aminophylline (25)	4	82.9±2.3	−8.8
Aminophylline (25) + Salbutamol (0.5)	5	60.6±4.7[d]	20.5
Cromoglycate (10)	5	54.8±2.3[e]	28.1

a All drugs were injected i.v. immediately before antigen injection. *b* Mean±S.E.M. *c* Since 0.9 % NaCl injected i.v. had no effect on the skin histamine content, non-treated rats were used as controls. Significantly different from the control value by Student's *t*-test : *d* $p < 0.01$, *e* $p < 0.001$.

salbutamol (0.5 mg/kg) were administered i.v. together immediately before the challenging injection, a significant inhibition of the decrease in the histamine content was observed.

Cromoglycate (10 mg/kg) given i.v. immediately before the antigen injection had a more marked inhibitory effect on the PCA-induced histamine decrease than the combination of aminophylline and salbutamol.

Effect of drugs on the PCA-induced decrease in the skin histamine content : experiments with the 15-mm steel punch. When the 15-mm steel punch was used to obtain skin samples, the percent decrease in the skin histamine content in the PCA site of the control animals was slightly smaller than when the 12-mm steel punch was used (Table 2).

Differing from the results obtained with the smaller punch, salbutamol (0.5 mg/kg) injected i.v. immediately before the antigen challenge had a marked and significant preventive effect on the PCA-induced skin histamine decrease (Table 2). Salbutamol (1.0 mg/kg) administered s.c. 30 min before the antigen challenge had almost no effect. Aminophylline (75 mg/kg) administered in the same way had no significant effect either.

When aminophylline (75 mg/kg) and salbutamol (0.5 mg/kg) were administered s.c. 30 min and i.v. immediately before the antigen injection, respectively, the decrease in the skin histamine content in the PCA site was markedly inhibited. This effect of the combination of aminophylline and salbutamol was about equal to the sum of the effects of the two drugs administered separately.

When aminophylline (75 mg/kg) and salbutamol (1.0 mg/kg) were injected s.c. together 30 min before the antigen challenge, a significant inhibition of the PCA-induced decrease in the skin histamine levels was observed. This combined effect was far greater than the sum of the effects of the two drugs administered

TABLE 2. EFFECT OF SALBUTAMOL, AMINOPHYLLINE AND CROMOGLYCATE ON THE PCA-INDUCED DECREASE IN THE SKIN HISTAMINE CONTENT AS DETERMINED BY EXCISING THE SKIN SAMPLES WITH A STEEL PUNCH OF 15 MM IN DIAMETER

Drug (mg/kg)	Route of administration[a]	No. of rats	% Decrease in the histamine content[b]	% Inhibition
Control[c]	—	12	66.3±2.5	—
Salbutamol (0.5)	i.v.	4	40.3±3.7[e]	39.2
Salbutamol (1.0)	s.c.	4	65.0±3.8	2.0
Aminophylline (75)	s.c.	4	61.2±3.3	7.7
Aminophylline (75) + Salbutamol (0.5)	s.c. i.v.	6	34.7±4.8[e]	47.7
Aminophylline (75) + Salbutamol (1.0)	s.c. s.c.	9	45.5±3.2[e]	31.4
Cromoglycate (10)	i.v.	10	47.9±3.4[d]	27.8

a Drugs were injected i.v. immediately or s.c. 30 min before antigen injection. *b* Mean± S.E.M. *c* Non-treated rats. Significantly different from the control value: *d* $p < 0.005$, *e* $p < 0.001$.

TABLE 3. EFFECT OF SALBUTAMOL, AMINOPHYLLINE AND CROMOGLYCATE ON THE PCA-INDUCED DYE LEAKAGE IN THE RAT SKIN

Drug (mg/kg)	Route of administration[a]	No. of rats	Amount of Evans blue accumulated (μg)[b]	% Inhibition
Control[c]	—	4	279±25	—
Salbutamol (1.0)	s.c.	3	46± 5[e]	83.5
Aminophylline (75)	s.c.	4	193±12[d]	30.8
Aminophylline (75) + Salbutamol (1.0)	s.c. s.c.	4	60± 7[e]	78.5
Cromoglycate (10)	i.v.	4	223±35	20.1

a Drugs were injected s.c. 30 min or i.v. immediately before antigen injection. *b* Mean± S.E.M. *c* Non-treated rats. Significantly different from the control value: *d* $p < 0.05$, *e* $p < 0.01$.

alone.

Cromoglycate (10 mg/kg) injected i.v. immediately before the antigen administration had almost the same degree of inhibitory effect on the PCA-induced decrease in the skin histamine levels, irrespective of the diameter of the steel punch used (Tables 1 and 2).

Effect of drugs on the PCA-induced dye leakage. In the non-treated control rats, an intense blue spot of 15-20 mm in diameter was observed 30 min after the challenging injection. The amount of Evans blue which accumulated in the skin area of 15 mm in diameter around the antigen-injected site was about 280 μg (Table 3).

Salbutamol (1.0 mg/kg) administered s.c. 30 min before the antigen injection markedly inhibited the PCA-induced dye leakage. In this case, a faint blue spot less than 5 mm in diameter was observed. This effect of salbutamol (1.0 mg/kg,

s.c.) was in striking contrast with its failure to inhibit the PCA-induced decrease in the skin histamine levels. Aminophylline (75 mg/kg, s.c.) also had a significant inhibitory effect on the dye leakage, although this effect was far less marked than that of salbutamol (1.0 mg/kg, s.c.). The effect of the combination of the two drugs did not surpass the effect of salbutamol administered alone. The effect of cromoglycate (10 mg/kg) administered i.v. immediately before the antigen injection was not significant, probably because the individual values varied so greatly.

DISCUSSION

Kurose (9) reported that BALB/c mice produced high titers of IgE antibodies when they were immunized with a minute amount of OA, using Al $(OH)_3$ as an adjuvant. Mouse IgE antibodies are able to induce a heterologous PCA reaction in rats, but other classes of mouse antibodies are devoid of the ability to sensitize the rat skin (10). Rat peritoneal mast cells sensitized *in vitro* with IgE-rich mouse antiserum release histamine in response to an antigen (9, 11).

An attempt has already been made to estimate the *in vivo* inhibitory effect of drugs on the histamine release which occurs in the local anaphylactic reaction, employing passive peritoneal anaphylaxis (PPA) as a test system (12). In this method, peritoneal washings are collected shortly after the antigen injection (i.p.), and the amount of histamine in the supernatant of the peritoneal washings is determined. The advantage of the present method over the PPA method is that the intensity of the tissue reaction is expressed in terms of the percent decrease in the tissue histamine content.

Goose and Blair (1) reported that cromoglycate inhibited the degranulation of mast cells in the site of IgE-mediated PCA. The results of our experiments are consistent with their findings. To estimate the effect of drugs on the mast cell response in the PCA site, the decrease in the tissue histamine content is easier to determine and more quantitative than is the degranulation of these cells.

In the present experiments, the percentage of the skin histamine decrease in the PCA site diminished with the increase in the diameter of the excised skin. This means that the effect of drugs on a less intense release reaction can be detected by employing a punch with a larger diameter. In fact, although salbutamol (0.5 mg/kg) administered i.v. immediately before the antigen injection had no significant inhibitory effect on the skin histamine decrease in the PCA site excised with a punch of 12-mm in diameter, it had a marked suppressive effect when the punch diameter was 15-mm. Therefore, for testing antiallergic drugs, the 15-mm punch seems preferable to the 12-mm punch. It has been observed *in vitro* that an intense histamine release reaction is generally more resistant to the inhibitory action of drugs compared with a weak reaction (13-16). In the present experiments, a fivefold dilution of the mouse antiserum was used to sensitize the rat skin. By the same immunization procedure as used in the present

study, Kurose (9) obtained from BALB/c mice anti-OA sera which showed very high PCA-titers (640-1280) when tested in the rat. Therefore, the antiserum used in the present experiments might be too strong to test the drug effect. The optimal dilution of antiserum to estimate the drug inhibition of the PCA-induced histamine release needs to be elucidated in further studies.

It has been demonstrated that intravenously administered salbutamol inhibits the *in vivo* histamine release from rat mast cells induced by PPA (12, 17). This is consistent with our results. Theophylline inhibits the antigen-induced *in vitro* histamine release from rat mast cells (18-20). It also inhibits the antigen-induced elevation of plasma histamine levels in allergic subjects (21). However, there has been almost no convincing evidence of the theophylline inhibition *in vivo* of the anaphylactic histamine release from rat mast cells.

Butchers *et al.* (22) reported that intraperitoneally administered salbutamol in doses up to 2.0 μg/kg was ineffective on the PPA-induced histamine release. In their experiments, salbutamol had only a modest inhibitory effect even at high concentrations on the antigen-induced *in vitro* histamine release from rat peritoneal mast cells. On the other hand, binding studies (23, 24) showed the presence of β-receptors on these cells. At present, the reason for the low sensitivity *in vitro* of these cells to salbutamol is not clear. Taylor and Sheldon (25) reported that heat-labile factor(s), which enhanced the sensitivity of rat peritoneal mast cells to the degranulation-inhibiting effect of isoproterenol, were released from chopped rat lung tissues when these tissues were incubated with isoproterenol. Therefore, it is difficult to say from the present experiments whether the inhibition by salbutamol of the PCA-induced histamine release is solely due to its direct effect on cutaneous mast cells.

Theophylline inhibits cyclic AMP phosphodiesterase of mast cells *in vitro* (26) and increases cyclic AMP levels in these cells (26, 27). However, rather high concentrations are necessary for such effects. Recently, questions have been raised whether theophylline inhibits the histamine release from mast cells by the inhibition of phosphodiesterase of these cells (20, 28, 29). In the present experiments, a synergistic inhibitory effect on the PCA-induced decrease in the skin histamine content was observed when salbutamol and aminophylline were administered together. Since adenylate cyclase is activated by the β-receptor stimulation and aminophylline is an inhibitor of phosphodiesterase, the observed synergistic effect might be due to an increase in intracellular cyclic AMP levels. However, the identity of cells in which such a change takes place remains unclear.

The leakage of protein-bound dye into the PCA site was much more sensitive to the action of salbutamol than was the decrease in the skin histamine content. Therefore, the salbutamol inhibition of the dye leakage seems to be largely due to its inhibitory effect on the vascular permeability increase. The anti-inflammatory action of salbutamol has already been demonstrated (30). The present results show that aminophylline injected s.c. alone has a slight inhibitory

effect on the vascular permeability increase. It is also evident that cromoglycate has no such effect.

In conclusion, the present study demonstrated that the determination of the skin histamine levels in the PCA site is useful to estimate the drug effect on the *in vivo* histamine release from cutaneous mast cells. It also showed that the inhibition of the leakage of protein-bound dye is an insufficient index of the drug effect on the anaphylactic cell response.

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