ANTIALLERGIC ACTION OF 6-ETHYL-3-(1H-TETRAZOL-5-YL) CHROMONE (AA-344) ON IMMEDIATE HYPERSENSITIVITY REACTION IN RATS

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Abstract—A newly synthesized compound, 6-ethyl-3-(1H-tetrazol-5-yl)chromone (AA-344) given intravenously or orally inhibited considerably the 72-hr passive cutaneous anaphylaxis (72-hr PCA) induced by IgE in rats. The antiallergic action of AA-344 was neither due to the antihistamine or antiserotonin effect nor was it mediated via adrenergic mechanisms. The results obtained in a double sensitization with two IgE antibodies suggest that AA-344 may not impair antigen-antibody combination but probably prevents the release of chemical mediators including histamine. This assumption was supported by observation that AA-344 inhibited a reduction in the skin histamine content caused by the 72-hr PCA, without effect on the compound 48/80-induced histamine reduction. AA-344 also partially inhibited the IgGa-mediated 3-hr PCA in rats. These results indicate that the inhibitory action of AA-344 on the immediate hypersensitivity reactions is due to prevention of the release of chemical mediators from the mast cells, by acting on some process in sequential events leading to the mediator release following antigen-antibody combination.

Atopic diseases including allergic bronchial asthma are considered to be provoked by an excess of a reaginic antibody production in response to various allergens (1, 2). The discovery of disodium cromoglycate (DSCG), which has been demonstrated to inhibit the immediate hypersensitivity (type I allergic) reactions in experimental models in vivo and in vitro, has brought about significant advances in the last decade in the treatment of asthma (3, 4, 5). However, its clinical effectiveness is limited as inhalation is the only route of ingestion. In recent years, baicalein having the same chromone structure as DSCG, which is the aglycone of baicalin present in Scutellaria baicalensis Georg, and its water-soluble derivative, disodium baicalein-6-phosphate have been found to inhibit allergic reactions in experimental models (6). In our continued research to develop a more potent orally effective antiallergic agent, a number of chromone derivatives were synthesized and tested biologically.

Chemical structure of AA-344
One of these derivatives, 6-ethyl-3-(1H-tetrazol-5-yl)chromone (AA-344) was found to be highly effective, when given orally, in inhibiting passive cutaneous anaphylaxis (PCA) after a 72-hr latent period in rats. This paper describes the pharmacological properties and a possible mode of action of AA-344.

MATERIALS AND METHODS

**Animals and administration of chemicals:** Male Sprague-Dawley rats weighing 250–300 g (8-week old) were used. Adrenalectomy or splenectomy in rats was performed by usual procedures. The adrenalectomized animals were kept on saline added tap water for drinking for 6 days before use.

Unless otherwise stated, AA-344, disodium cromoglycate (DSCG, Fisons), promethazine, isoproterenol or propranolol was administered i.v. or p.o. before antigen challenge in conscious rats. In some rats under ether anesthesia, AA-344 was given intraduodenally or intraileally, or intragastrically following pylorus-ligation, prior to antigen challenge. AA-344 was dissolved in a solution of equimolar sodium bicarbonate for i.v. injection and suspended in 5% gum arabic solution for p.o. administration. All other chemicals were dissolved in saline.

**Antisera:** Rat anti-egg albumin antiserum (anti-EA) was prepared by the method of Mota (9). Rats were immunized by giving i.m. into each hind limb 1 mg of EA (Difco) in 1 ml saline divided equally, and i.p. 1 ml suspension of $2 \times 10^{10}$ killed *Bordetella pertussis* (Takeda). Twelve days later serum was obtained and pooled. The titer of the antiserum, i.e. the highest dilution inducing PCA in rats after a 72-hr latent period was 1:16.

Rat anti-dinitrophenylated-ascaris extract antiserum (anti-DNP-asc) was prepared by the method of Okumura and Tada (10). Five days after splenectomy, rats were immunized by giving s.c. into each of four footpads a 1 ml mixture of 1 mg protein of ascaris extracts (asc) and $10^{10}$ killed *Bordetella pertussis* divided equally into four parts. After 5 days, 0.5 mg of DNP-asc was given s.c. into the back. Six days after the last injection, serum was obtained and pooled. The rat 72-hr PCA titer of the antiserum was 1:32.

The presence of IgE in these antisera was examined for the following characteristics: persistence of PCA activity with a latent period in excess of 72-hr, loss of PCA activity by heating at 56°C for 1 hr or by alkylating with iodoacetamide following reduction with 2-mercaptoethanol (11), and chromatographic patterns similar to those of human IgE on Sephadex G-200 and DEAE-cellulose column chromatography.

Rat anti-EA antiserum containing IgGa was prepared from hyperimmune rats by the method of Orange et al. (12). Rats were immunized with a 1 ml mixture of equivolume of 2 mg EA in saline and Freund’s complete adjuvant (Difco) (day 0), in which 0.1 ml was injected into each of four footpads and the remaining 0.6 ml was given i.p. On day 7, a 1 ml mixture containing 1 mg EA and 0.5 ml of Freund’s complete adjuvant was divided into five parts, each of which was given i.m. into both thighs and s.c. at three sites on the back, respectively. On days 28 and 35, 0.1 mg EA in a 0.1 ml saline divided equally was given i.d. at two sites on the back. On day 43, serum was obtained, pooled and stored after
heating at 56°C for 4 hr. The antiserum with the rat 3-hr PCA titer of 1:16 was found to contain no IgE as it did not induce 72-hr PCA at a 2-fold dilution. The antiserum diluted 4-fold with saline was used for experiments.

**IgE-mediated 72-hr PCA reaction:** Rats were sensitized by injecting i.d. on the back 0.05 ml aliquots of IgE-containing anti-EA or anti-DNP-asc. After a 72-hr latent period, the animals were given i.v. 1 ml of saline containing 5 mg EA or 2 mg DNP-asc, together with 10 mg Evans blue (Wako). Thirty min after the antigen challenge, the skin was exfoliated and the intensity of PCA reaction was evaluated by expressing the size of a wheal stained with dye as the product (mm²) of the longest diameter and the line perpendicular to it. Anti-EA and anti-DNP-asc, both diluted 4-fold with saline, produced the 72-hr PCA with 248 ± 6 (n=54) and 258 ± 9 (n=3) in wheal sizes, respectively.

**IgGa-mediated 3-hr PCA reaction:** Rats were sensitized with IgGa-containing anti-EA as described in the 72-hr PCA. After a 3-hr latent period, the animals were given 1 ml of saline i.v., which contained 5 mg EA and 10 mg Evans blue, and 30 min later the PCA intensity was evaluated. The antiserum diluted 4-fold with saline showed the 3-hr PCA with 231 ± 6 mm² (n=9) in wheal size.

**Local bluing reactions by histamine, serotonin and others in rats:** In groups of 5 rats, each animal was given i.d. the following substances in 0.05 ml saline at different sites on the back: histamine 2HCl (Wako) 50 μg; serotonin creatinine sulfate (Wako) 10 μg; bradykinin (Osaka Univ.) 10 μg; trypsin (Difco) 100 μg; compound 48/80 (Sigma) 10 μg; polymyxin B (Pfizer) 10 μg. Immediately, AA-344 or DSCG was given i.v. together with 10 mg Evans blue. The wheal size was examined 30 min later. Effect of promethazine HCl (Yoshitomi) was also tested by p.o. administration 2 hr before injections of the above substances plus the dye.

**Histamine content in rat skin:** As described above, the rats sensitized with IgE-containing anti-EA were challenged with i.v. injection of EA and Evans blue 72 hr later. These animals also were given an i.d. injection of compound 48/80 at the site apart from that injected with antiserum on the back, immediately before antigen challenge. Skin specimens of 15 mm in diameter were taken, using a punching device, from the dye stained sites and untreated site as control. Each skin specimen was weighed, chopped into small pieces and suspended in 5 ml of Tyrode solution. Histamine in the skin tissue was extracted by boiling for 5 min, purified by an Amberlite IRP-64 column chromatography and determined by the fluorophotometric method of Shore et al. (13). The procedure provided more than 90% recovery of histamine added to a tissue suspension.

**Double sensitization with two IgE antibodies:** This experiment was performed according to the method of Orr et al. (14). IgE-containing anti-EA and anti-DNP-asc were used at dilutions capable of inducing the 72-hr PCA with approx. 250 mm² in the wheal size. Groups of 3 rats were sensitized with anti-EA, anti-DNP-asc and a mixture of both antisera, given i.d. at different sites on the back. Seventy-two hr later the animals were challenged with either 5 mg EA or 2 mg DNP-asc, followed by a second challenge with one of these antigens and Evans blue 4 hr later. AA-344 or DSCG was administered i.v. immediately before
the first antigen challenge.

**Statistical analysis:** The results obtained were expressed as means \(\pm\) standard errors (S.E.). Student's t-test was used to make a statistical comparison between the groups.

**RESULTS**

**Inhibitory effect of AA-344 on the 72-hr PCA**

Antigen challenge after a 72-hr latent period in rats sensitized with i.d. administration of anti-EA in the concentration range of 1 to 32-fold dilutions provoked an antiserum dose-dependent PCA as indicated by an increase in the wheal size. This antiserum-PCA response curve was shifted to the right, i.e. inhibition, after i.v. administration of AA-344 at 0.31 and 1.25 mg/kg immediately before antigen challenge. When antiserum at 4-fold dilution was used, AA-344 and DSCG in varying doses given i.v. immediately before antigen suppressed the 72-hr PCA in a dose dependent manner (Fig. 1a). The ID50 values, i.e. the doses required to reduce the PCA response by 50% were 0.28 mg/kg for AA-344 and 1.30 mg/kg for DSCG: the former compound was 4.6 times as potent as the latter.

AA-344 given p.o. 5 min before antigen challenge also suppressed the 72-hr PCA in a dose dependent manner (Fig. 1b) and had an ID50 value of 5.4 mg/kg. By contrast, DSCG at p.o. doses as high as 20 and 100 mg/kg was inactive. Promethazine at 2.5–10 mg/kg given p.o. 2 hr before antigen challenge also had a quantitative inhibition of the 72-hr PCA.

To determine the main site of absorption in the gastrointestinal tract, AA-344 at 0.63–5 mg/kg was given intragastrically 5 min before antigen to the pylorus-ligated rats and sham-operated rats. Inhibitory activity of AA-344 in the former rats was approx. half that in the latter. Furthermore, AA-344 at 10 mg/kg given intraduodenally or intraileally 5 min before antigen was also effective in PCA inhibition. These results indicate that AA-344 is rapidly absorbed from almost all of the gastrointestinal tract.

**FIG. 1.** Effects of AA-344 and DSCG on the rat 72-hr PCA. AA-344 or DSCG was given i.v. immediately before antigen challenge (a) and p.o. 5 min before antigen (b). The control wheal sizes (mm²) were 248 ± 6 for (a) and 243 ± 9 for (b), respectively. Each numeral in parenthesis represents number of animals.
FIG. 2. Duration of the inhibitory action of AA-344 on the rat 72-hr PCA. AA-344 or DSCG was given i.v. (a) and AA-344 was given p.o. (b) at various times before antigen challenge. The control wheal sizes (mm²) were 248±19 (n=3) for (a) and 210±6 (n=25) for (b), respectively. Each point represents the average of three animals.

Duration of the inhibitory effect

AA-344 was given i.v. or p.o. at various times prior to antigen challenge in order to determine duration of the inhibitory effect. Maximum activity was obtained when the compound was given i.v. immediately before antigen or p.o. within 15 min before antigen. An inhibitory effect was not manifested when AA-344 was given i.v. 60 min before (Fig. 2a). A similar time-course of the inhibitory effect was obtained with DSCG. AA-344 at 10 or 50 mg/kg given p.o. had a relatively long-lasting action (Fig. 2b).

AA-344 at 1.25 mg/kg given i.v. 0.5 and 1 min after antigen challenge was effective as indicated by 70-90% inhibition, but was no longer active 5 min later.

Modification of the inhibitory action of AA-344 by pretreatment

To determine whether or not pretreatment with AA-344 or DSCG had any influence on the own inhibitory effect in the 72-hr PCA, each compound was given i.v. to sensitized rats 1 hr prior to the second dose of either compound, by the same route, and was immediately followed by challenge with the antigen. This pretreatment time is considered to be sufficient

| TABLE 1. Effects of pretreatment with AA-344 on inhibition of the rat 72-hr PCA by second administration of AA-344 |
|---------------------------------------------------------------|
| First AA-344 without antigen (mg/kg, i.v.) | % Inhibition of 72-hr PCA | Second AA-344 with 5 mg EA \( ^a \) |
|---------------------------------------------|-----------------|-----------------|
| 0                                           | 8 \( ^b \)      | 15              | 99              |
| 0.01                                        | 18              | 54              | 99              |
| 0.1                                         | 21              | 37              | 99              |
| 1.0                                         | 24              | 53              | 53              |

\( ^a \) One hour was allowed to elapse between the i.v. administrations of first AA-344 and second AA-344 with antigen (EA).

\( ^b \) Mean value of three rats (12 spots).
to rule out the possibility of a direct effect of the compound on the PCA. Pretreatment with AA-344 at 0.01, 0.1 or 1 mg/kg clearly enhanced the inhibitory effect of the second dose of the same compound at 0.01 and 0.1 mg/kg. The maximum inhibitory effect of the second 1 mg/kg dose of AA-344 was not altered by pretreatment with the above two lower doses, but was reduced by approx. 50% after pretreatment with a high dose at 1 mg/kg (Table 1). Similar results were obtained with 0.1, 1 and 10 mg/kg of DSCG. Furthermore, a cross reactivity between AA-344 and DSCG was observed. Pretreatment with AA-344 at 1.25 mg/kg reduced the inhibitory effect of DSCG at 2.5 mg/kg from 94% inhibition to 58%. In the reverse situation, pretreatment with DSCG at 50 mg/kg also reduced the ability of AA-344 at 1.25 mg/kg to inhibit the PCA from 99% inhibition to 49%.

Modification of the inhibitory action of AA-344 by adrenalectomy and ß-adrenergic antagonist

We investigated whether the PCA inhibitory action of AA-344 was mediated via adrenal hormones or by ß-adrenergic mechanisms. The wheal sizes (mm², n = 3) in the 72-hr PCA were 186 ± 19 in normal rats, 204 ± 9 in sham-operated rats and 148 ± 26 in adrenalectomized rats: PCA reactivity tended to be somewhat reduced by adrenalectomy. However, similar dose-PCA inhibition curves for AA-344 at 0.16, 0.31 and 0.63 mg/kg given i.v. before the antigen were obtained in both adrenalectomized and sham-operated rats. Furthermore, the inhibitory effect of AA-344 was not influenced by i.v. administration of a ß-adrenergic antagonist, propranolol at 1 or 4 mg/kg 15 min before antigen, in which the inhibitory effect of isoproterenol in a dose of 10 µg/kg i.v. was completely blocked.

Effect of AA-344 on local bluing reactions by histamine, serotonin and other substances

Neither AA-344 nor DSCG in a dose of 20 mg/kg i.v. had any significant effect on the bluing reactions induced by i.d. administrations of histamine, serotonin, bradykinin, trypsin, compound 48/80 and polymyxin B (Table 2). On the other hand, promethazine given p.o. in a dose of 5 mg/kg inhibited considerably the bluing responses to histamine and compound 48/80.

Effect of AA-344 on the histamine content in rat skin

Reduction of the histamine content in the sensitized skin resulting from the mediator release in the 72-hr PCA reaction was significantly prevented by 5 mg/kg of AA-344 given i.v., immediately before the antigen challenge. Such a reduction in the levels of histamine was suppressed to some extent but not significantly with 1 mg/kg of AA-344 or 5 mg/kg of DSCG. There was no significant effect of AA-344 or DSCG in reducing histamine content so induced by compound 48/80 (Table 3).

Effect of AA-344 on the skin sensitization with IgE antibody

To examine the influence of AA-344 on binding of IgE antibody to receptors on mast cells, rats were sensitized i.d. with 0.05 ml of 4-fold diluted anti-EA containing AA-344 or with anti-EA alone 5 min after p.o. administration of AA-344. The PCA reaction provoked by antigen 72 hr later was not significantly altered by AA-344 as follows: the wheal sizes (mm²) in groups of 5 rats were 230 ± 8 in control, 217 ± 6 and 214 ± 6 in the groups given i.d. 0.5 and 5 µg of AA-344, and 221 ± 5 and 222 ± 5 in the groups given p.o. 10 and 50 mg/kg.
Table 2. Effects of AA-344, DSCG and promethazine on the dermal dye leakage due to histamine, serotonin, bradykinin, trypsin, compound 48/80 and polymyxin B in rat skin

| Compound | Dose       | No. of rats | Dye leakage, mm² |        |        |        |        |        |
|----------|------------|-------------|------------------|-------|-------|-------|-------|-------|
|          |            |             | Histamine 50 μg  | Serotonin 10 μg | Bradykinin 10 μg | Trypsin 100 μg | Compound 48/80 10 μg | Polymyxin B 10 μg |
| Exp. 1   |            |             |                  |                   |                   |                   |                   |                   |
| Saline   | —          | 5           | 223 ± 13         | 592 ± 31          | 167 ± 9           | 223 ± 15          | 211 ± 16          | 224 ± 21          |
| AA-344   | 20 mg/kg i.v. | 5          | 232 ± 20         | 590 ± 25          | 159 ± 7           | 205 ± 14          | 217 ± 14          | 240 ± 11          |
| DSCG     | 20 mg/kg i.v. | 5          | 213 ± 11         | 564 ± 42          | 185 ± 7           | 202 ± 12          | 229 ± 15          | 234 ± 11          |
| Exp. 2   |            |             |                  |                   |                   |                   |                   |                   |
| 5% Gum arab. | —         | 5           | 253 ± 11         | 645 ± 35          | 177 ± 12          | 293 ± 40          | 292 ± 19          | —                 |
| Promethazine | 5 mg/kg p.o. | 5          | 87 ± 21***       | 517 ± 33*         | 108 ± 15**        | 200 ± 18          | 174 ± 39*         | —                 |

*P < 0.05; **P < 0.01; ***P < 0.001 vs. 5% gum arabic group.

AA-344 or DSCG with Evans blue was given i.v. immediately before i.d. administration of histamine, serotonin or others, and promethazine was given p.o. 2 hr before.
of AA-344, respectively.

**Effect of AA-344 on the PCA in double sensitization with two IgE antibodies**

In an attempt to determine at what stage in the PCA reaction AA-344 was effective, the experiments were performed using a double sensitization technique according to the method of Orr et al. (14). The results in Table 4 show that PCA reactions were induced by a single challenge with either EA or DNP-asc and by two sequential challenges with either the same or a different antigen in the rats sensitized with either anti-EA or anti-DNP-asc alone and a mixture of both antisera. In the two sequential antigen challenge system, there was a 4 hr interval between the first and second challenges, so that the effect of AA-344 could

### Table 3. Effects of AA-344 and DSCG on reduction in skin histamine content induced by 72-hr PCA and compound 48/80 in rats

| Compound | Dose (mg/kg, i.v.) | No. of rats | Histamine content, µg/g tissue | △A | △B  |
|----------|-------------------|------------|--------------------------------|----|-----|
|          |                   |            | Untreated 72-hr PCA Compound 48/80 |
| Saline   | -                 | 6          | 26.9 ± 2.2 18.3 ± 1.7* 16.5 ± 2.0* | 8.6 ± 1.9 | 10.4 ± 3.0 |
| AA-344   | 1                 | 6          | 24.1 ± 1.8 21.3 ± 1.2 — | 2.9 ± 2.0 | —    |
| AA-344   | 5                 | 6          | 24.0 ± 2.9 24.7 ± 2.7 17.2 ± 1.9 | —0.7 ± 3.0 | 6.9 ± 3.2 |
| DSCG     | 1                 | 5          | 26.6 ± 2.5 21.3 ± 1.2 — | 5.3 ± 3.0 | —    |
| DSCG     | 5                 | 6          | 25.3 ± 1.4 22.6 ± 1.7 18.7 ± 1.1* | 2.8 ± 1.9 | 6.6 ± 1.1 |

△A: Untreated site - 72-hr PCA site. △B: Untreated site - compound 48/80 site.
*P<0.05 vs. untreated site. §P<0.05 vs. saline group.

### Table 4. Effect of AA-344 on the rat IgE-mediated PCA reaction using the double sensitization technique

| Sensitizing antisera | Antigen challenge | PCA, mm² |
|----------------------|-------------------|----------|
|                      | First             | Second   |
| Anti-EA              | —                 | EA       | 275 ± 9  |
| Anti-EA              | EA                | EA       | 0        |
| Anti-EA              | EA + AA-344       | EA       | 0        |
| Anti-DNP-asc         | —                 | DNP-asc  | 258 ± 9  |
| Anti-DNP-asc         | DNP-asc           | DNP-asc  | 0        |
| Anti-DNP-asc         | DNP-asc + AA-344  | DNP-asc  | 0        |
| Anti-EA + Anti-DNP-asc| —                | EA       | 245 ± 19 |
| Anti-EA + Anti-DNP-asc| EA                | EA       | 0        |
| Anti-EA + Anti-DNP-asc| EA                | DNP-asc  | 52 ± 6   |
| Anti-EA + Anti-DNP-asc| EA + AA-344       | EA       | 0        |
| Anti-EA + Anti-DNP-asc| EA + AA-344       | DNP-asc  | 270 ± 15 |
| Anti-EA + Anti-DNP-asc| —                 | DNP-asc  | 286 ± 16 |
| Anti-EA + Anti-DNP-asc| DNP-asc           | DNP-asc  | 0        |
| Anti-EA + Anti-DNP-asc| DNP-asc           | EA       | 2 ± 2    |
| Anti-EA + Anti-DNP-asc| DNP-asc + AA-344  | DNP-asc  | 0        |
| Anti-EA + Anti-DNP-asc| DNP-asc + AA-344  | EA       | 215 ± 6  |

Three rats were used in each experiment.
AA-344 at a dose of 1.25 mg/kg was given i.v. immediately before the first antigen challenge and the second antigen without AA-344 was given 4 hr later.
be excluded at the second antigen challenge.

In the skin sites sensitized with the individual antibody, anti-EA to EA challenge and anti-DNP-asc to DNP-asc challenge produced the PCA of $275 \pm 9$ and $258 \pm 9$ mm², respectively. However, the second challenge with the same antigen produced no PCA reaction, as the antibody had already been inactivated by reacting with the antigen in the first challenge and the tissue became unresponsive to the second challenge of antigen, i.e. a desensitization of the tissue occurred. Loss of the PCA response to the second challenge with the same antigen was also observed when AA-344 at 1.25 mg/kg, a dose sufficient to inhibit completely the PCA, was given at the first antigen challenge, indicating that a desensitization of the tissue still occurred in the presence of AA-344.

In the skin sites sensitized with a mixture of anti-EA and anti-DNP-asc, a challenge with EA or DNP-asc provoked the PCA of $245 \pm 19$ or $286 \pm 16$ mm². In the two sequential challenge system, the second challenge with the same antigen gave no PCA response regardless of the presence or absence of AA-344 during the first antigen-antibody combination. This phenomenon was similar to that seen previously in the skin sites sensitized with the individual antibody. Following the first challenge with either EA or DNP-asc in the absence of AA-344, the second challenge with the alternative antigen provoked weak PCA, as shown by the wheal sizes of $2 \pm 2$ mm² for EA and $52 \pm 6$ mm² for DNP-asc. However, when AA-344 was given at the first antigen challenge, a typical PCA response to the second challenge with the alternative antigen appeared. Similar results were obtained with 5 mg/kg of DSCG.

**Effect of AA-344 on the 3-hr PCA**

AA-344 at 5 and 20 mg/kg given i.v. immediately before antigen challenge inhibited slightly but significantly the 3-hr PCA induced by anti-EA containing IgGa in rats. A similar effect was observed with the same dose of DSCG (Table 5).

| Compound | Dose (mg/kg, i.v.) | No. of rats | 3-hr PCA, mm² | % Inhibition |
|----------|-------------------|-------------|---------------|--------------|
| Saline   | —                 | 9           | $231 \pm 6$   | —            |
| AA-344   | 5                 | 10          | $173 \pm 8^{***}$ | 25           |
| AA-344   | 20                | 10          | $154 \pm 16^{***}$ | 33           |
| DSCG     | 5                 | 10          | $172 \pm 9^{***}$ | 26           |
| DSCG     | 20                | 10          | $148 \pm 8^{***}$ | 36           |

***P<0.001 vs. saline group.

**DISCUSSION**

A newly synthesized chromone compound, AA-344 given i.v. immediately before antigen challenge was shown to be 4.6 times as active as DSCG in inhibiting the IgE-mediated 72-hr PCA in rats. The action attained a maximum level when AA-344 or DSCG was given immediately before antigen, but disappeared almost completely when given 1 hr prior to the test. In contrast to DSCG, AA-344 was also effective when given p.o. and had rapid
onset and relatively long duration of action. A maximum effect was produced even when given p.o. 5 min before antigen challenge and such a rapid onset of action was considered to be due to a rapid absorption of the compound from not only the intestine but also the stomach, as based on results obtained by intragastrical administration after pylorouligation and by the intraintestinal route. Furthermore, AA-344 given i.v. within 1 min after antigen challenge also inhibited effectively the 72-hr PCA, suggesting that the compound would remain effective even in the presence of the process of antigen-antibody combination and/or an early stage in sequential events leading to release of chemical mediators. A paradoxical, transient potentiation of the rat PCA described by Rosenthal et al. (15) with Wy-16,922 and DSCG was not observed with AA-344.

It is generally accepted that the IgE-mediated PCA is produced by pharmacological actions of chemical mediators such as histamine and serotonin released from the sensitized mast cell and resulting from a combination of antigen with cell-bound IgE antibody (5, 16, 17). Since an antihistaminic agent, promethazine markedly prevented the PCA in our present experiment, histamine seems to be a more effective mediator in the 72-hr PCA. AA-344 did not modify the local bluing reaction induced by histamine, histamine liberators such as compound 48/80 and polymyxin B, serotonin or other substances. These results indicate that the PCA inhibitory action of AA-344 is not due to blocking of either the histamine and serotonin receptors or an other non-specific mechanism responsible directly for the increase in capillary permeability.

The anaphylactic release of histamine from rat mast cells (18, 19), human leukocytes (20) and human lung (21, 22) is inhibited by epinephrine, isoproterenol or cyclic AMP phosphodiesterase inhibitors, in association with a rise in the intracellular cyclic AMP levels. The effects of catecholamines are antagonized by a β-adrenergic antagonist, propranolol (20, 22). Involvement of adrenal hormones or β-adrenergic mechanisms in the PCA inhibitory action of AA-344 can be excluded as the action was not modified by adrenalectomy or propranolol. Whether or not AA-344 alters the intracellular levels of cyclic nucleotides has not been reported. AA-344 clearly prevented a reduction in the histamine content in the skin where the 72-hr PCA reaction occurred, but was ineffective in case of a 48/80-induced decrease in histamine content. These findings suggest that AA-344 inhibits specifically the anaphylactic histamine release. This idea was given further support by the results in the in vitro experiments with isolated rat peritoneal mast cells (23).

AA-344 had no inhibitory effect on the IgE binding to the receptors on mast cells, evidenced by the finding that a passive sensitization of the skin mast cell with IgE in the presence of high concentration of AA-344 was followed by a manifestation of the normal PCA reaction. The site of action was further elucidated from the results obtained in the experimental system of a double sensitization with two different IgE antibodies, anti-EA and anti-DNP-asc and two sequential challenges with the corresponding antigen, EA or DNP-asc. In these experiments, an effective dose of AA-344 was administered only during the initial process of antigen-antibody combination. When the same antigen was challenged twice in sequence, the second antigen challenge produced no PCA regardless of the presence
or absence of AA-344 at the initial antigen challenge. These results suggest that the initial antigen-antibody combination takes place even in the presence of AA-344 and results in a desensitization of the tissue to the second antigen, although a different antibody in the sensitized tissue may remain active. Furthermore, when the rat was challenged with one antigen in the absence of AA-344, followed by another antigen, only a weak PCA reaction was provoked by the second antigen challenge in the site sensitized doubly with the antibodies. In this case, the antigen-antibody combination may take place with the second antigen challenge, but this combination does not proceed to a PCA reaction because chemical mediators to be released in the sensitized tissue have already been depleted by the preceding antigen-antibody combination. However, the presence of AA-344 during the period of antigen-antibody combination preserved completely the PCA responsiveness of the tissue to the second challenge with another antigen, indicating that the mast cells retained an intact function to release mediators. These findings strongly suggest that AA-344 prevents the release of chemical mediators from the doubly sensitized tissue by acting on some process in the sequence following the initial antigen-antibody combination.

There were interesting but complicated dual phenomena: enhancement and/or reduction, i.e. tachyphylaxis, of the ability of AA-344 to inhibit the PCA was observed after pretreatment with the same compound. Either enhancement or tachyphylaxis depended upon the amounts in both predosing and second dosing in antigen challenge: the PCA inhibitory effect of low doses (0.01 and 0.1 mg/kg) of AA-344 was enhanced by predosing with either low or even a high dose (1 mg/kg), and in contrast the effect of 1 mg/kg was reduced by predosing of 1 mg/kg. A cross tachyphylaxis between AA-344 and DSCG was observed. This suggests that AA-344 is likely to share a mechanism in common with that of DSCG. Similar dual phenomena or tachyphylaxis in other antiallergic agents have also been reported (15, 24, 25, 26, 27). If we attempt to explain these dual phenomena by quoting the speculations of the aforementioned authors, two sites can be proposed for the action of AA-344: one is a membrane site which may be partially altered in the conformation, by exposure to large amounts of AA-344, so that the second low dose of AA-344 can enter into the cell while the high dose does not. Another is probably some enzyme in the cell relating directly or indirectly to the mediator release and action of AA-344, which may become more susceptible or accessible to the second dose of AA-344 after exposure to any amount of AA-344.

IgGa and IgE are immunochemically distinct antibodies with different functional characteristics but homocytotropic, and both induce the mediator release from the mast cell (28, 29). Furthermore, a common receptor or closely interacting receptors have been suggested as such would interfere with each other in the rat PCA reaction and in the mediator release from the sensitized rat peritoneal mast cell provoked by antigen (30, 31). In the present study, AA-344, like DSCG, suppressed partially the rat 3-hr PCA induced by antiserum containing IgGa, although 100 times the dose of AA-344 was required to inhibit the IgE-mediated 72-hr PCA. This observation is supported by our recent result that AA-344 inhibits the IgGa-mediated histamine release from the isolated rat peritoneal mast cell (23). It has been demonstrated that DSCG partially inhibits the IgGa (7S_r2)-mediated PCA in
rats (32, 33), and that such consists of two parts, an early immediate reaction inhibited by DSCG and a late reaction resistant to it (34). This may explain the partial inhibition of AA-344 on the 3-hr PCA.

In conclusion, AA-344 is an orally effective compound which inhibits considerably IgE-mediated anaphylaxis and also partially IgGa-mediated anaphylaxis by preventing the release of chemical mediators in a manner similar to that seen with DSCG.

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