EFFECT OF 6-ETHYL-3-(1*H*-TETRAZOL-5-YL)CHROMONE (AA-344) ON THE IMMEDIATE AND DELAYED HYPERSENSITIVITY REACTIONS

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Abstract—Effects of 6-ethyl-3-(1*H*-tetrazol-5-yl)chromone (AA-344) on the experimental models of the type I-IV allergic reactions were studied in comparison with those of disodium cromoglycate (DSCG), dexamethasone and other agents. AA-344 showed inhibitory effects on the homologous passive cutaneous anaphylaxis and the passive systemic anaphylaxis in guinea pigs. However, it had only a slight or little effect on the Forssman shock in guinea pigs, the complement-dependent cytolysis of mast cells in rats, the Arthus reaction in guinea pigs and the tuberculin reaction and the contact sensitivity in mice. DSCG was less effective, using these experimental models. Dexamethasone showed a suppressive effect on the type III and IV allergic reactions. The results indicate that AA-344 selectively suppresses the type I allergic reaction.

We previously reported that 6-ethyl-3-(1*H*-tetrazol-5-yl)chromone (AA-344) by either i.v. or p.o. administration markedly suppressed the IgE-mediated hypersensitivity due to inhibition of the release of chemical mediators from the mast cells (1, 2). The mode of action of AA-344 *in vivo* and *in vitro* on the type I allergic reaction, is similar to that of disodium cromoglycate (DSCG) (3, 4).

The inhibitory effect of DSCG on the late asthmatic reaction provoked by inhalation of antigen in patients with asthma, which is classified as the type III allergic reaction (3, 5), was reported by some groups (5, 6), while such could not be confirmed by another group (7). There is apparently no documentation concerning the effect of DSCG on the experimental model of type III allergic reaction in animals. The present study was an attempt to determine whether AA-344 or DSCG, an inhibitor of chemical mediator release from mast cells, has a suppressive effect not only on type I allergic reaction but also on the type II, III and IV allergic reactions, as compared with effects of dexamethasone and antihistamines, in experimental models.

MATERIALS AND METHODS

Animals: The animals used were male JCL:ICR mice (25–31 g), male C57BL/6 mice (21–34 g), male BALB/c mice (19–27 g), male Sprague-Dawley rats (250–350 g), Hartley guinea pigs of either sex (280–450 g) and male albino rabbits (2.5–4.0 kg).

Chemicals: The following chemicals were used: bovine- γ -globulin (BGG, Wako), bovine serum albumin fraction V (BSA, Wako), egg albumin (EA, Difco), Freund's complete

adjuvant (FCA, Difco), Freund's incomplete adjuvant (FIA, Difco), killed *Bordetella pertussis* (Takeda), killed *Mycobacterium tuberculosis* Aoyama B strain (Tokyo University), purified protein derivatives of tuberculin (PPD, Nippon BCG), sheep red blood cells (Nihon Seibutsu-zairyo Center), promethazine HCl (Yoshitomi), cyproheptadine HCl (Merck), dexamethasone phosphate (Takeda), disodium cromoglycate (DSCG, Intal®, Fisons). AA-344 and diphenhydramine were synthesized in the Takeda Chemical Labs. Benzyl-penicilloyl-BGG (BPO₅₀BGG) and benzyl-penicilloyl-BSA (BPO₃₂BSA) were prepared according to the method of Horiuchi and Shibata (8).

Homologous passive cutaneous anaphylaxis (PCA) in guinea pigs: Guinea pig anti-BPO₅₀BGG antisera containing IgE-like or IgG antibody were prepared according to the method of Levine *et al.* (9). PCA titers for the IgE-like and IgG antibodies were 1:64 and 1:128, respectively. Guinea pigs were sensitized passively by giving intradermally (i.d.) 0.05 ml of the 16-fold diluted antiserum containing IgE-like antibody on the left side of the back. Eight days later, these animals were also sensitized by i.d. administration of the 32-fold diluted antiserum containing IgG antibody, on the right side of the back. The PCA was induced by an i.v. challenge with 1 mg of BPO₃₂BSA and 10 mg of Evans blue dissolved in 1 ml of a physiological saline 3 hr after the sensitization with IgG. The animals were sacrificed by bleeding 30 min after the challenge of the antigen and the skin was exfoliated. The intensity of PCA was estimated by measuring the area of the dye-stained wheal, as a product (mm²) of the longest and correspondingly perpendicular diameters.

Heterologous PCA in guinea pigs: Rabbit anti-EA antiserum (anti-EA) was prepared according to the method of Koda *et al.* (10). Groups of 5 guinea pigs were sensitized by giving i.d. 0.05 ml of the 100-fold diluted antiserum. Three hr later the animals were challenged i.v. with 5 mg of EA and 10 mg of Evans blue.

Passive systemic anaphylaxis (PSA) in guinea pigs: Groups of 16–27 female guinea pigs were passively sensitized by giving i.v. 0.2 ml of the rabbit antiserum described above. Twenty-four hr later the PSA was provoked by administering i.v. the lethal dose (0.51 mg/kg) of EA calculated according to the method of Uda (11). The severity of the PSA shock was graded on the following scales: symptom I, nose licking and rubbing, fur ruffling and weakness due to diminished muscle tone; II, labored breathing, sneezing or coughing, retching and micturition; III, defecation, convulsion, prostration and death.

Active systemic anaphylaxis (ASA) in mice: Groups of 19–20 male JCL:ICR mice were sensitized by giving i.p. 1 mg of EA and 2×10^{10} organisms of killed *Bordetella pertussis* according to the method of Greig *et al.* (12). Fifteen days after the sensitization, the ASA was provoked by giving i.v. 1 mg of EA and the mortality was recorded 30 min later.

Passive Schultz-Dale reaction in guinea pig ileum: Isolated guinea pig ileum of about 10 cm was incubated with rabbit anti-EA (containing 100 µg antibody nitrogen) in 10 ml of Tyrode's solution at 37°C for 1 hr. The sensitized ileum was suspended in a 20-ml bath containing the Tyrode's solution warmed at 37°C and aerated. For observation of the passive Schultz-Dale reaction, the contractile response of the ileum to antigen (2 mg of EA) was recorded isotonicly with a resting tension of 0.5 g. The response to histamine

(2.5×10^{-7} M) was also determined as standard.

Hemolysis of sheep red blood cell (SRBC) caused by rabbit anti-Forssman antigen antiserum and complement (in vitro) and Forssman shock in guinea pigs (in vivo): Forssman antigen and rabbit anti-Forssman antigen antiserum were prepared according to the method of Nishioka (13). *In vitro*: complement-dependent hemolysis of SRBC was determined by the method of Mayer (14). *In vivo*: Forssman shock in guinea pigs was provoked by giving i.v. 0.3 ml of the antiserum. The anaphylactoid symptoms observed within 3 min after injection were weakness, labored breathing, snivelling, prostration and death. Complement activity (CH50: 50% hemolytic unit of complement) in the serum of guinea pigs before the injection of the antiserum and immediately after death was determined according to the method of Mayer (14).

Complement-dependent cytolysis of rat peritoneal mast cells: Peritoneal cells were harvested from the peritoneal cavity of rats and the mast cells (RMC) were purified according to the method of Sullivan *et al.* (15), and rabbit anti-RMC antiserum (rabbit anti-RMC) was prepared by the method of Yiu and Froese (16). Experimental procedures of the complement-dependent cytolysis of the RMC were performed as described by Valentine *et al.* (17) as follows. *In vitro*: purified RMC (10^6 cells) was sensitized with 1 ml of the 100-fold diluted anti-RMC for 15 min at 37°C. The sensitized RMC (10^5 cells), rinsed and suspended in a mast cell medium (MCM) (15), was incubated with 0.1 ml of the dialysed normal rabbit serum as a source of complement for 60 min at 37°C in a total volume of 1 ml. After centrifugation, histamine in the supernate was determined by the fluorometric method of Shore *et al.* (18). Lactate dehydrogenase (LDH) activity was determined using the LDH_B Test Wako kit. The degree of cytolysis of RMC was determined by a staining test with trypan blue (17). *In vivo*: rats were given i.p. 1 ml of the 30-fold diluted rabbit anti-RMC. Four hr later, the peritoneal cells were harvested from the rat peritoneal cavity. Total histamine content in the peritoneal cells was used as a parameter of the cytolysis of RMC *in vivo*.

Arthus reaction in guinea pigs: The passive Arthus reaction was induced according to the method of Benacerraf and Kabat (19). Male guinea pigs were given i.v. 1.1 ml of rabbit anti-EA (1 mg of antibody nitrogen) and, immediately thereafter, given i.d. into both flanks 0.2 ml of a EA solution (0.07 mg of antigen nitrogen) or saline. The intensity of the reaction was indicated in the legend of Table 6.

Tuberculin reaction in mice: Male C57BL/6 mice were sensitized by administering s.c. an emulsion of 0.05 ml of killed *Mycobacterium tuberculosis* in saline (500 µg) and 0.05 ml of FIA. Fourteen days later, the mice were challenged with 5 µg of PPD dissolved in 0.04 ml of distilled water into left footpad. The thickness of the right footpad injected with saline and the left footpad injected with PPD was measured with a Dial Thickness Gauge (Peacock) 24 hr after the challenge.

Contact sensitivity to 2,4-dinitro-1-fluorobenzene (DNFB) in mice: Contact sensitivity to DNFB was tested according to the method of Phanuphak *et al.* (20). Male BALB/c mice were sensitized by applying 20 µl of 0.5% DNFB in acetone-olive oil (4:1) on the shaved skin

surface of the abdominal wall for two days. Five days after the second sensitization, the mice were challenged with application of 10 μ l of 0.2% DNFB on the dorsal surface of each ear. The ear thickness was measured by using the Dial Thickness Gauge immediately before and 24 hr after the challenge.

Statistical analysis: The values obtained were expressed as means $\pm$ standard errors (S.E.). Student's *t*-test and chi-square test were used for a statistical comparison between the groups.

RESULTS

Effects on homologous and heterologous PCA in guinea pigs: Control wheal sizes of the homologous 8-day PCA induced by IgE-like antibody and the homologous 3-hr PCA induced by IgG antibody were 190 ± 6 and 217 ± 19 mm², respectively. AA-344 (20 mg/kg i.v.) given immediately before the antigen challenge significantly suppressed both the PCAs. DSCG (5 and 20 mg/kg i.v.) showed no significant inhibitory effects (Table 1). Similar effects of both compounds were also observed when the 8-day and 3-hr PCAs were provoked in the animals sensitized with the 4-fold higher concentration of the antisera. The heterologous PCA showing wheal size of 181 ± 27 mm² was not inhibited by AA-344 or DSCG (5 and 20 mg/kg i.v.) given immediately before the antigen challenge (Table 1).

Effects on PSA in guinea pigs and ASA in mice: The antigen challenge to guinea pigs sensitized passively with i.v. administration of rabbit anti-EA, resulted in symptoms of grade III in 24 and death in 23 out of 27 animals, within 30 min after the antigen challenge. AA-344 (5 mg/kg i.v.) given immediately before the antigen challenge decreased the mortality, though it did not alleviate the symptoms. However, a high dose of AA-344 (20 mg/kg i.v.) markedly alleviated the symptoms in 10 out of 18 animals and prevented death in all animals (Table 2). The same doses of DSCG had no effect on the anaphylactic shock. Promethazine (1 mg/kg i.v.) markedly reduced symptoms of the PSA. The intravenous challenge of antigen (EA) to the sensitized mice resulted in death of 19 out of 20 mice within 30 min (Table 2). The ASA was not alleviated by AA-344 or DSCG (5 and 20 mg/kg i.v.) given 5 min before the challenge. Cyproheptadine (1 mg/kg i.v.) significantly reduced the mortality due to the ASA.

Effects on passive Schultz-Dale reaction in guinea pigs: Before and after each test, the

TABLE 1. Effects of AA-344 and DSCG on homologous 8-day and 3-hr PCA reactions and on heterologous 3-hr PCA reaction in guinea pigs

Drugs	Dose (mg/kg i.v.)	Homologous PCA, mm ²			Heterologous PCA, mm ²	
		No. of animals	8-day	3-hr	No. of animals	3-hr
Saline	—	6	190 ± 6	217 ± 19	5	181 ± 27
AA-344	5	5	193 ± 14	193 ± 12	5	173 ± 31
	20	6	$141 \pm 20^{**}$	$134 \pm 15^{**}$	5	169 ± 21
DSCG	5	5	178 ± 24	213 ± 29	5	167 ± 22
	20	6	202 ± 21	246 ± 17	5	178 ± 13

^{**}P<0.01 vs. saline control group.

TABLE 2. Effects of AA-344, DSCG, promethazine and cyproheptadine on passive systemic anaphylaxis (PSA) in guinea pigs and on active systemic anaphylaxis (ASA) in mice

Drugs	Dose (mg/kg i.v.)	PSA in guinea pigs				ASA in mice	
		Symptoms, No. of animals				Mortality	
		0	I	II	III	Died/Total	Died/Total
Saline	—	0	0	3	24	23/27	19/20
AA-344	5	0	0	3	13	8/16*	19/19
	20	2	8	7	1	0/18**	18/19
DSCG	5	0	0	3	13	13/16	18/20
	20	0	2	1	13	13/16	17/19
Promethazine	1	0	3	12	1	0/16**	—
Cyproheptadine	1	—	—	—	—	—	2/9*

* $P < 0.05$; ** $P < 0.01$ vs. saline control group.

TABLE 3. Effects of AA-344, DSCG and diphenhydramine on passive Schultz-Dale reaction in guinea pigs

Drugs	Conc. (M)	N	Schultz-Dale reaction ^{a)}	
			Saline	Drug
AA-344	10^{-5}	5	101 ± 10	94 ± 12
	10^{-4}	5	101 ± 10	91 ± 11
	10^{-3}	5	101 ± 10	75 ± 14
DSCG	10^{-5}	5	102 ± 9	99 ± 13
	10^{-4}	5	102 ± 9	91 ± 11
	10^{-3}	5	102 ± 9	115 ± 11
Diphenhydramine	10^{-7}	3	112 ± 8	113 ± 12
	10^{-6}	3	103 ± 12	$2 \pm 1^{***}$

a) % contraction height, compared with the histamine response (2.5×10^{-7} M).*** $P < 0.001$ vs. saline treated group.

responsiveness of the isolated guinea pig ileum passively sensitized with rabbit anti-EA was checked with histamine as a spasmogen. A challenge with an optimal concentration of antigen (10^{-4} g EA/ml) provoked a rapid contractile response followed by a slow contractile response of the sensitized ileum. The height of the rapid contraction was submaximal, that is similar to the contractile response to histamine (2.5×10^{-7} M). In this study, a decrease in the height of the rapid contractile response was used as a measure of evaluation. AA-344 (10^{-5} and 10^{-4} M) added 2 min before the challenge had no effect on the passive Schultz-Dale reaction (Table 3), but with a high concentration of AA-344 (10^{-3} M) a slight inhibition of the reaction was seen. On the contrary, DSCG (10^{-5} to 10^{-3} M) showed no inhibitory activity. Diphenhydramine (10^{-6} M) significantly depressed the reaction.

Effects on complement-dependent cytolysis of SRBC or RMC and on hypotonic cytolysis of SRBC (in vitro): The degree of the complement-dependent hemolysis of SRBC *in vitro* under the standard assay condition with rabbit anti-Forssman antigen antiserum was 47.2

TABLE 4. Effects of AA-344 and DSCG on complement-dependent cytolyse of SRBC and RMC and on hypotonic cytotoxicity of SRBC (*in vitro*)

Drugs	Conc. (M)	Complement-dependent cytotoxicity				Hypotonic cytotoxicity	
		N	SRBC % hemolysis	N	RMC % histamine release	N	SRBC % hemolysis
None	—	5	47.2±3.8	5	57.7±3.0	3	53.0±1.0
AA-344	3×10 ⁻⁴	4	43.9±4.3	5	57.4±5.4	3	48.5±2.3
	1×10 ⁻³	4	31.5±4.0**	5	60.0±4.8	3	43.3±4.8
	3×10 ⁻³	4	11.0±2.3***	5	38.1±6.5*	3	30.9±2.2***
DSCG	3×10 ⁻⁴	4	44.5±4.6	5	50.3±4.4	3	49.6±3.0
	1×10 ⁻³	4	36.2±3.9	5	35.8±3.0**	3	45.5±3.1
	3×10 ⁻³	4	7.8±3.2***	5	30.8±4.1**	3	28.7±6.3*

*P<0.05; **P<0.01; ***P<0.001 vs. non-treated group. The histamine release induced by rabbit anti-RMC antiserum and complement and the spontaneous histamine release were 57.7±3% (n=5) and 3.6±0.8% (n=5) of the total histamine content in the mast cells (15.3±0.6 µg/10⁶ cells, n=5), respectively. The total histamine content was determined from the histamine released by boiling the sensitized cells for 15 min.

±3.8% (Table 4). Both AA-344 and DSCG (0.3, 1 and 3×10⁻³ M) added simultaneously with complement showed a concentration-dependent inhibition of the hemolysis of SRBC. The degree of the complement-dependent cytotoxicity of the purified RMC with rabbit anti-RMC expressed in terms of the released histamine, the released lactate dehydrogenase activity and the number of RMC stained with trypan blue were 59.8, 62.2 and 49.7%, respectively. Under the same condition, AA-344 (3×10⁻³ M) added simultaneously with complement significantly inhibited the cytotoxicity (Table 4). DSCG (1 and 3×10⁻³ M) also showed a significant inhibition. Experiment on the hypotonic cytotoxicity of SRBC shown in Table 4 was performed to evaluate a non-selective membranous stabilization such as that of anti-inflammatory drugs. The concentration of sodium chloride required to induce a hypotonic hemolysis of SRBC by approx. 50% was 65 mM. The high concentrations of AA-344 and DSCG similar to those used in the inhibition of the complement-dependent cytotoxicity of SRBC and RMC *in vitro* suppressed the NaCl-hypotonic cytotoxicity (Table 4). These results suggest that the inhibitory action of AA-344 and DSCG on the complement-dependent cytotoxicity may be related, at least partly, to the stabilizing activity on membrane.

Effects on Forssman shock in guinea pigs and on cytotoxicity of RMC in rats in vivo: The i.v. administration of rabbit anti-Forssman antigen antiserum in guinea pigs (n=8) provoked the Forssman shock. All of the animals died within 3 min and the complement activity (CH50) was reduced by approx. 50% (Table 5). AA-344, DSCG (5 and 20 mg/kg i.v.) and dexamethasone (10 mg/kg i.v.) given immediately before the challenge had no effect on the shock or reduction of the complement activity. The degree of the complement-dependent cytotoxicity of RMC provoked by the i.p. administration of rabbit anti-RMC was expressed in terms of the reduction in the histamine content of the peritoneal cells harvested. The average histamine contents of the peritoneal cells in the rats treated with normal rabbit serum and rabbit anti-RMC 4 hr before harvest of the cells were 40.9±1.1 µg (n=3) and 16.3±4.2

μg ($n=6$) per rat, respectively. AA-344 and DSCG (5 and 20 mg/kg i.v.) given immediately before the challenge had no effect, and dexamethasone showed a significant suppression of the cytolysis (Table 5).

Effects on Arthus reaction in guinea pigs: The Arthus reaction with hemorrhage and edema was provoked by the i.d. administration of antigen (EA) in guinea pigs passively sensitized with the i.v. administration of rabbit anti-EA. Eleven out of 12 animals treated with saline produced the Arthus reaction with the degree of severity + or higher, which was defined as positive (Table 6). In the histological study with hematoxylin-eosin staining, infiltration of polymorphonuclear leukocytes and monocytes around the blood vessels in the lesion was observed. When the degree of severity of $\pm$ to ++++ was converted into numeral scores of 1 to 5, the average score in the saline-treated animals was 3.3. AA-344 (5 and 20 mg/kg i.v.) given immediately before the antigen challenge slightly reduced the

TABLE 5. Effects of AA-344, DSCG and dexamethasone on Forssman shock in guinea pigs and on cytolysis of RMC in rats given i.p. rabbit anti-RMC antiserum

Drugs	Dose (mg/kg i.v.)	N ^{a)}	Forssman shock in guinea pigs							N	Cytolysis of RMC
			Mortality (%)	Survival time (sec)	Complement activity, CH50 ^{b)}			De- crease (%)	Histamine content of peritoneal cells ($\mu\text{g}/\text{rat}$)		
					Before challenge	After death					
Saline	—	8	100	121 $\pm$ 43	139.3 $\pm$ 11.6	74.1 $\pm$ 4.7	47	6	16.3 $\pm$ 4.2		
AA-344	5	8	100	112 $\pm$ 45	137.8 $\pm$ 5.1	58.7 $\pm$ 10.4	57	3	14.4 $\pm$ 2.1		
	20	8	100	147 $\pm$ 40	149.6 $\pm$ 12.5	80.0 $\pm$ 7.7	47	3	19.4 $\pm$ 8.7		
DSCG	20	7	100	108 $\pm$ 14	167.6 $\pm$ 23.7	88.5 $\pm$ 13.8	47	3	20.1 $\pm$ 5.1		
Dexamethasone	10	8	100	121 $\pm$ 1	124.0 $\pm$ 10.8	72.0 $\pm$ 7.8	42	3	26.5 $\pm$ 2.7*		

a) N: Number of animals. b) CH50: 50% hemolytic unit of complement. *: $P<0.05$

TABLE 6. Effects of AA-344, DSCG and dexamethasone on passive Arthus reaction in guinea pigs

Drugs	Dose (mg/kg. i.v.)	No. of animals	Severity of Arthus reaction							Mean score	Positive ($\geq +$)
			++++	+++	++	+	$\pm$	—			
Saline	—	12	4	1	2	4	1	0	3.3	11/12	
AA-344	5	11	3	1	2	2	1	2	2.7	8/11	
	20	11	2	1	1	2	3	2	2.2	6/11	
DSCG	5	11	1	2	0	6	1	1	2.4	9/11	
	20	11	2	3	1	5	0	0	3.2	11/11	
Dexamethasone	10	10	0	0	1	4	4	1	1.5	5/10	

Grade: + + + +; A confluent hemorrhage of 10 mm or more in length (longest axis) with severe edema at 2 hr, and a hemorrhagic discoloration of 10 mm in length and moderate to severe edema at 24 hr. + + +; A petechial hemorrhage of 15 mm in length with moderate to severe edema at 2 hr, and a discoloration of 5 to 10 mm in length with moderate edema at 24 hr. ++; A petechial hemorrhage of 10 to 15 mm in length with moderate edema at 2 hr, and a discoloration of 5 mm in length with mild to moderate edema at 24 hr. +; A mild edema, petechial hemorrhage of 5 to 10 mm in length at 2 hr, and the reaction was still visible at 24 hr. $\pm$; A petechial hemorrhage of less than 5 mm in length with slight or no edema at 2 hr. Negative at 24 hr. —; No reaction.

TABLE 7. Effects of AA-344, DSCG and dexamethasone on tuberculin reaction and contact sensitivity in mice

Drugs	Dose (mg/kg, i.p.)	No. of mice	Tuberculin reaction	Contact sensitivity
			Footpad swelling $\times 10^{-2}$ mm	Increase in ear thickness $\times 10^{-2}$ mm
5% gum arabic	—	8	118.8 $\pm$ 9.0	7.9 $\pm$ 0.8
AA-344	5	8	107.6 $\pm$ 12.3	6.9 $\pm$ 0.6
	20	8	136.1 $\pm$ 22.2	7.2 $\pm$ 0.6
DSCG	5	8	136.4 $\pm$ 10.3	6.8 $\pm$ 0.7
	20	8	117.1 $\pm$ 11.1	6.9 $\pm$ 0.7
Dexamethasone	5	8	63.8 $\pm$ 5.9**	3.9 $\pm$ 0.5**

Drugs were administered i.p. 30 min before the challenge. Swelling of footpads measured 24 hr after the challenge was expressed as the difference in thickness between the right footpad (saline) and left footpad (PPD). **: $P < 0.01$

number of positive responses and the score. The same doses of DSCG hardly affected the Arthus reaction. Dexamethasone (10 mg/kg i.v.) reduced considerably but not significantly the degree of severity and the score of the Arthus reaction.

Effects on tuberculin reaction and contact sensitivity in mice: The tuberculin reaction in mice sensitized with killed *Mycobacterium tuberculosis* and FIA was provoked by the challenge of PPD. The footpad swelling 24 hr after the challenge was approx. 120×10^{-2} mm as measured using a thickness gauge (Table 7). AA-344 and DSCG (5 and 20 mg/kg i.p.) given 30 min before the challenge showed no effect, whereas dexamethasone (5 mg/kg i.p.) significantly suppressed the reaction. In non-sensitized mice, the increment of the ear thickness at 24 hr after the application of DNFB was 0.9×10^{-2} mm. However, in the sensitized mice, the increment of the ear thickness 24 hr after the challenge of DNFB was 7.9×10^{-2} mm (Table 7). Dexamethasone (5 mg/kg i.p.) given 30 min before the challenge of DNFB significantly suppressed the reaction, but AA-344 and DSCG (5 and 20 mg/kg i.p.) had no effect on the contact sensitivity to DNFB.

DISCUSSION

Our previous work showed that AA-344 as well as DSCG markedly inhibited the IgE-mediated PCA in rats (1) and that the antiallergic action is due to the prevention of the chemical mediator release from the mast cells (2). In the work reported herein, the homologous 8-day PCA in guinea pigs, which is reportedly induced by the antiserum containing IgE-like antibody (9, 21), was significantly inhibited by AA-344. The antiserum used in the present experiment showed the following IgE-like characteristics: presence of the PCA activity for more than 8 days, a partial but significant thermolability at 56°C for 4 hr, and a complete inactivation by alkylation with iodoacetamide following reduction with 2-mercaptoethanol. These properties were similar to those of human IgE antibody characterized by Ishizaka and Ishizaka (22), the rat IgE antibody reported by Stechshulte *et al.* (23), and the guinea pig antibody observed by Levine *et al.* (9). The role of mast cells on the 8-day PCA in guinea pigs has also been considered to be the same as that on the IgE-mediated

PCA in rats (24). All these findings taken together suggest that the mode of the inhibitory action of AA-344 on the 8-day PCA in guinea pigs was the same as that on the 72-hr PCA in rats. On the other hand, the homologous 3-hr PCA in guinea pigs provoked by the antiserum containing IgG antibody (γ_1 -antibody) was also inhibited by AA-344, similar to that on the IgGa-mediated PCA in rats reported previously (1). However, DSCG in doses of 5 and 20 mg/kg i.v. showed no inhibitory effect on both the 8-day PCA and 3-hr PCA in guinea pigs. Taylor and Roitt (25) reported that DSCG reduced the PCA reaction induced by IgE-like antibody in guinea pigs. However, Martin (26) detected no inhibitory action of DSCG on the IgE-like antibody-induced PCA in guinea pigs, such being in agreement with our present results. Furthermore, no inhibitory effect of DSCG on the 3-hr PCA induced by γ_1 -antibody in guinea pigs was observed by Cox *et al.* (4), Taylor and Roitt (25), and Lopez and Bloch (27).

The *in vitro* complement-dependent cytolysis of SRBC or RMC, as type II allergic reactions, was significantly inhibited by AA-344 and DSCG at the concentrations up to 10^{-3} M, but the *in vivo* reactions of the Forssman shock in guinea pigs and the cytolysis of RMC in rats were not affected by these compounds. The inhibitory action of AA-344 on the *in vitro* type II allergic reaction may be related, at least in part, to the non-specific membranous stabilization similar to that seen with anti-inflammatory drugs, because AA-344 possessed a preventive action regarding the cytolysis of SRBC, as induced by hypotonic treatment *in vitro*.

Regarding the passive Arthus reaction in guinea pigs as the experimental model of the type III allergic reaction, AA-344 was found to slightly alleviate the symptoms characterized by hemorrhage. The Arthus reaction in rabbits was reported to proceed in two stages, i.e., the maximum dye leakage in the lesions was attained at both 30 min and 5 hr after the challenge with antigen (28). The early reaction was reduced by antihistamines while the late reaction was not, suggesting the involvement of histamine in development of the early reaction. Cochrane (29) suggested the participation of IgE, IgG, basophilic leukocytes or mast cells in the early stage of the immune complex disease (serum sickness) in rabbits. Koda *et al.* (30) demonstrated a moderate inhibitory action of N(3',4'-dimethoxycinnamoyl)-anthranilic acid on the Arthus reaction in rabbits. This compound was also reported to possess inhibitory activity on the release of histamine from the mast cells and the anti-inflammatory activity on the rat carrageenin edema (31). These observations suggest that increase in the permeability of vessels caused by type I allergic mechanism may be involved in the early stage of the Arthus reaction. If AA-344 actually suppresses the Arthus reaction, the mode of action may be associated with inhibition of the chemical mediator release from mast cells. However, DSCG did not inhibit the reaction, in the present study. The reported clinical effectiveness of DSCG on type III late asthmatic reaction provoked experimentally by inhalation of antigen (5-7), should therefore be examined further. On the other hand, Fishel (32) and Waksman (33) reported that treatment with an antihistamine failed to inhibit the rabbit homologous passive Arthus reaction. Therefore, a further study to analyze the immunological mechanism of the Arthus reaction may be a prerequisite to determine the

appropriateness of this reaction as an attempt to evaluate the antiallergic agent.

Increase in the vascular permeability in the delayed hypersensitivity as in the tuberculin reaction and the contact sensitivity is considered to be caused by lymphokines from the lymphocytes (34–36). However, Gershon *et al.* (37) proposed that the release of vasoactive amines from mast cells provided a modulation for the development of contact sensitivity and the footpad reaction in mice, as inhibited by pretreatment with reserpine. Additional evidence is required, however, for an accurate interpretation of the results as reserpine has various systemic effects. Neither AA-344 nor DSCG exerted an inhibitory action on the tuberculin reaction and the contact sensitivity to DNFB in mice.

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