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Antiallergic Effects of the Adrenergic β-Stimulant Trimetoquinol in Rats and Guinea Pigs

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Trimetoquinol (TMQ), like isoproterenol (Iso), inhibited not only the *in vitro* anaphylactic release of histamine from minced guinea pig lung tissue but also passive cutaneous anaphylaxis (PCA) in rats and guinea pigs and systemic anaphylaxis in guinea pigs. In these inhibitory effects, TMQ was more potent than Iso and other β-adrenergic stimulants (orciprenaline, salbutamol and terbutaline) *in vivo*, and 1000 times more active than disodium cromoglycate *in vitro*. In rat PCA, orally administered TMQ was fully effective as early as 5 min after administration and showed a longer duration of action than Iso. Furthermore, TMQ did not greatly affect the vascular permeability, unlike Iso, which markedly inhibited histamine-induced dye leakage on the rat skin, suggesting that the inhibition of PCA by Iso may be partly ascribed to this action on the capillary vessels, and therefore that the anti-anaphylactic activity of TMQ should be that much greater than that of Iso.

When rat mast cells were incubated with TMQ, the cellular level of adenosine-3′,5′-cyclic monophosphate (cAMP) was elevated. The inhibition of *in vitro* histamine release by TMQ and the elevation of cAMP in the mast cells by TMQ and Iso were both antagonized by propranolol, but not by practolol. These results indicate that TMQ and Iso inhibit the anaphylactic reactions by β-adrenergic mechanisms, probably of a β_2-type.

Keywords——antiallergic activity; trimetoquinol; isoproterenol; disodium cromoglycate; β-adrenergic receptor; cyclic AMP in mast cells; anaphylactic release of histamine; passive cutaneous anaphylaxis; systemic anaphylaxis; vascular permeability

Some forty years ago, Schild[2] observed that epinephrine inhibited antigen-induced histamine release from sensitized quinea pig lung tissue. Assem and Schild[3] later showed that this inhibition could be ascribed to adrenergic β-stimulation. In 1968, Lichtenstein and Margolis[4] reported that catecholamines and methylxanthines synergistically inhibited the anaphylactic release of histamine from atopic human leukocytes, and postulated that adenosine-3′,5′-cyclic monophosphate (cAMP) might play a role in the inhibition of histamine release. This observation was confirmed by other investigators, who studied the release of

1) Location: *2-2-50, Kawagishi, Toda-shi, Saitama, 335, Japan.*
2) H. Schild, *Quart. J. Exp. Physiol.*, **26**, 165 (1936).
3) *a*) E.S.K. Assem and H.O. Schild, *Nature* (London), **224**, 1028 (1969); *b*) E.S.K. Assem and H.O. Schild, *Brit. J. Pharmacol.*, **42**, 620 (1971).
4) L.M. Lichtenstein and S. Margolis, *Science*, **161**, 902 (1968).

histamine and other mediators (SRS–A and ECF–A) from various tissues of man and monkey.[3a,5]

The hypothesis that cAMP is a "second messenger" to regulate the release of allergic mediators[6] is supported by several lines of evidence.[4–7] In the earlier investigations, mixed cell populations were used to study the effect of β-stimulants on the elevation of cAMP. Recently it was reported that isoproterenol[8] and epinephrine[9] raised the cAMP level in purified mast cells, and that β-stimulation was involved in this process. Inhibition *in vivo* of anaphylactic mediator release by β-stimulants has also been reported in passive cutaneous anaphylaxis (PCA) and the Prausnitz–Küstner type reaction.[10]

In the present communication, we report on the inhibition of both *in vitro* and *in vivo* anaphylactic reactions and the elevation of cAMP in rat mast cells by the β-adrenergic broncholdilator trimetoquinol [TMQ; *l*-1-(3,4,5-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride].[11]

Materials and Methods

Chemicals——Ovalbumin (OA; five times recrystallized, ICN Pharmaceuticals), bacterial α-amylase (BA; Nagase Sangyo Co., Ltd.), isoproterenol hydrochloride (Iso; Nakarai Chemicals, Ltd.), Intal® (50% preparation of disodium cromoglycate (DSCG); Fujisawa Pharmaceutical Co., Ltd.) and Freund's complete adjuvant (Difco Laboratories) were commercial products. Orciprenaline sulfate, terbutaline sulfate and practolol hydrochloride were gifts from C.H. Boeringer Sohn, Astra Pharmaceutical Products, Ind. and Imperial Chemical Industries Ltd., respectively. Trimetoquinol (TMQ)[12] and salbutamol[13] were synthesized by Dr. E. Yamato and Mr. M. Wada, respectively, at the Organic Chemistry Laboratory of this company. Propranolol hydrochloride was prepared by extraction and recrystallization of a commercial preparation (Inderal®; Sumitomo Chemical Co., Ltd.). *Bordetella pertussis* vaccine which was used as an adjuvant for immunization of rats with the extract of *Ascaris suum* (As) was donated by the Research Institute for Microbial Diseases, Osaka University.

Animals——Male Sprague–Dawley rats (*ca.* 200 g in body weight), male albino guinea pigs (*ca.* 200 g for anaphylaxis and *ca.* 300 g for preparation of antisera) and male albino rabbits (*ca.* 3 kg) were purchased from Shizuoka Agricult. Coop. Assoc. Laboratory Animals and maintained on the laboratory chow before use.

Rabbit Anti-OA Antisera——Rabbits were immunized *i.m.* with 10 mg of OA in 1 ml of Freund's complete adjuvant containing 5 mg of tubercle bacilli. Four weeks later, the rabbits were reimmunized by the same procedure. Two or more weeks later, the sera were checked for antibody titer and the whole blood was withdrawn from the rabbits. The isolated sera were kept frozen until use.

5) *a*) T. Ishizaka, K. Ishizaka, R.P. Orange, and K.F. Austen, *J. Immunol.*, **106**, 1267 (1971); *b*) R.P. Orange, W.G. Austen, and K.F. Austen, *J. Exp. Med.*, **134**, 136s (1971); *c*) M. Kaliner, S.I. Wasserman, and K.F. Austen, *New Engl. J. Med.*, **289**, 277 (1973).
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Guinea Pig Anti-OA Antisera——Guinea pigs were immunized *i.m.* twice at an interval of 2 weeks with 6 mg of OA in Freund's complete adjuvant, and the antisera were obtained 2 weeks after the second immunization.

Rat Anti-As Antisera——Rats were immunized with the extract of As according to the method of Strejan and Campbell[14] except for the antigen doses (2 and 0.2 mg protein for the first and second immunizations, respectively). The sensitized rats were bled 2 weeks after the second immunization, and the sera were assayed for IgE in rat PCA. IgE was confirmed by its heat lability and long-lasting fixation on the rat skin.

Anaphylactic Release of Histamine——In most experiments, minced guinea pig lung tissue was sensitized *in vitro* with rabbit anti-OA antiserum and challenged with antigen according to the method of Monger and Schild.[15] Lungs of normal guinea pigs were removed and minced with scissors into pieces of *ca.* 2 mg in Tyrode's solution (pH 7.5). Three hundred mg of the minced tissue was incubated at 37° for 3 hr in 2 ml of 100 fold diluted rabbit anti-OA antiserum in Tyrode's solution. The sensitized tissue was washed, preincubated for 15 min with test compounds in 1.8 ml of Tyrode's solution and challenged by incubation with 10 μg of OA for 15 min in a final volume of 2 ml. The concentrations of test compounds were expressed as those in the final incubation mixture. At the end of incubation, the supernatants were boiled for 5 min and assayed for histamine. In some experiments, lungs of actively sensitized guinea pigs were used. The immunization procedure for guinea pigs was as described above.

Determination of Histamine——Histamine was determined by the spectrofluorometric technique of Shore *et al.*[16] Since TMQ interfered with the formation of the fluorophore, the deproteinized supernatants containing TMQ were washed three times with water-saturated *n*-butanol. For evaluation of the effect of test compounds, the amount of histamine released was expressed as a percentage of that of the control in triplicate experiments.

Determination of Cyclic AMP——The mast cell-rich fraction was obtained from the peritoneal cavity of male Sprague–Dawley rats according to the method of Sullivan *et al.*[9] The purity of mast cell preparations thus obtained was about 80%. Approximately 2.0×10^5 mast cells were incubated at 37° first with propranolol or practlol in the presence of 5×10^{-3} M theophylline for 2 min and then with TMQ or Iso for 1 min. The incubation mixture was boiled for 7 min and sonicated for 30 sec with an ultrasonic disrupter (model UR-200P, Tomy Seiko Co.). Cyclic AMP in the supernatant was determined by radioimmunoassay[17] using the Yamasa Cyclic AMP Assay Kit (Yamasa Syoyu Co., Ltd.). All the incubation mixtures were prepared in duplicate.

Rat PCA——Rats were passively sensitized by injecting *i.c.* on the back 0.05 ml each of 40, 80 and 160 fold dilutions in saline of rat anti-As antiserum, and 24 hr later challenged by injecting *i.v.* 1 ml of a solution containing 0.5 mg of As protein and 5 mg of Evans blue. The rats were treated with test compounds at various times (5 to 120 min) before the challenge. The magnitude of PCA response was expressed as the product of the largest diameter (cm) and its perpendicular diameter (cm) of the blueing area.

Guinea Fig PCA——Guinea pigs were passively sensitized by injecting *i.c.* on the back 0.05 ml of 300 fold diluted guinea pig and 1000 fold diluted rabbit anti-OA antisera, and 4 hr later challenged by injecting *i.v.* 1 ml of a solution containing 2 mg of OA and 5 mg of Evans blue. Fifteen min before the challenge, the guinea pigs were treated with test compounds.

Systemic Anaphylaxis——Guinea pigs were passively sensitized by injecting *i.v.* 1 ml of 25 fold diluted guinea pig anti-OA antiserum, and 24 hr later challenged by injecting *i.v.* 0.03 mg of OA in saline. Test compounds were administered along with the antigen solution (1.3 ml). The time in minutes from the challenge to the onset of anaphylactic responses (convulsion, spasmodic inspiration and termination of breathing) was recorded.

Histamine-induced Dye Leakage——Rats were treated with test compounds at various times (5 to 60 min) before *i.c.* injection of 0.05 ml of saline or a solution containing 1.7 or 5.0 μg of histamine hydrochloride. Evans blue (5 mg in 0.05 ml saline) was injected *i.v.* 10 min before the histamine injection. Dye leakage was assayed as described under "Rat PCA" 30 min after the histamine injection.

Results

Anaphylactic Release of Histamine from Minced Guinea Pig Lung Tissue Sensitized *in Vitro* with Rabbit Antisera

When the minced lung tissue of normal guinea pigs was sensitized *in vitro* with 100 fold diluted rabbit anti-OA antiserum and challenged by addition of various concentrations of

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15) J.L. Mongar and H.O. Schild, *J. Physiol.* (London), **150**, 546 (1960).
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OA or BA, the release of histamine was antigen-specific and depended on the concentration of the antigen (Fig. 1 A). Ten to 100 μg/ml of OA was optimal for anaphylactic histamine release under the conditions used, and OA at 1000 μg/ml was inhibitory. An optimal concen-

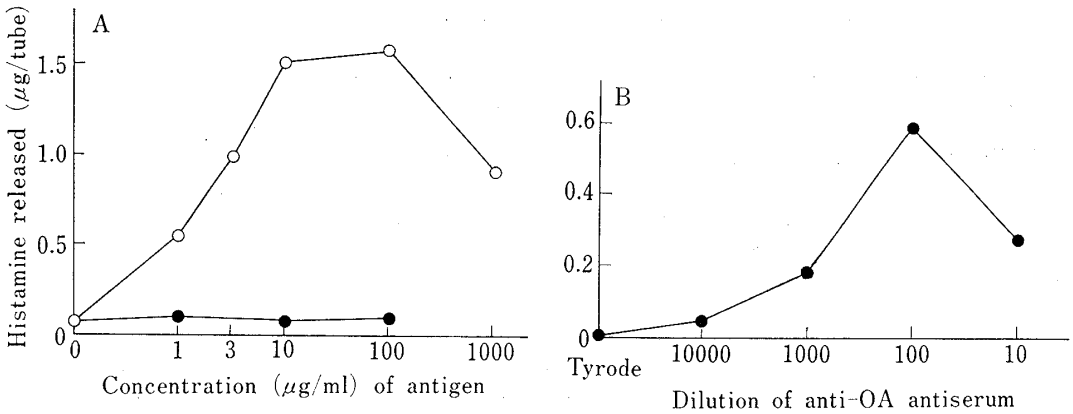

Fig. 1. Anaphylactic Release of Histamine from Minced Guinea Pig Lung Sensitized *in Vitro* with Rabbit Antiserum

A. 300 mg of minced lung tissue sensitized *in vitro* with 100 fold diluted rabbit anti-OA antiserum was challenged by incubation at 37° for 15 min with various concentrations of OA (○) or BA (●). B. The tissue sensitized with 10 to 10000 fold diluted antiserum was challenged with 10 μg/ml of OA. Net amounts of released histamine were measured.

tration of the antiserum was presented to the lung tissue for passive sensitization: incubation of the tissue with either more or less concentrated antiserum than the above dilution (100 fold) resulted in less histamine release on challenge with 10 μg/ml of the antigen (Fig. 1 B).

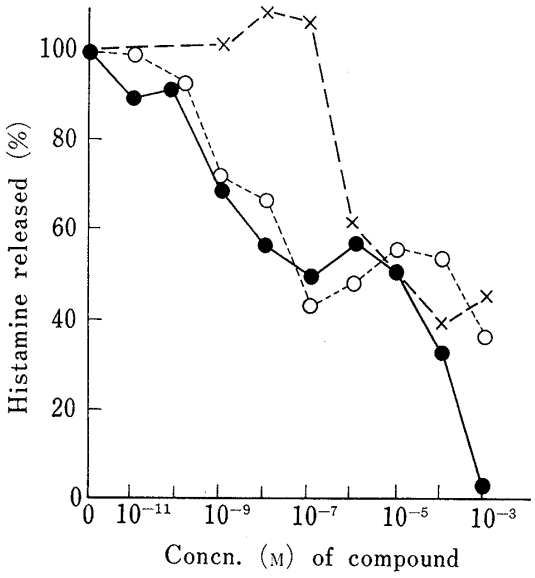

Fig. 2. Effects of TMQ, Iso and DSCG on Anaphylactic Release of Histamine from Lung Tissue Sensitized with Heterologous Antiserum

Sensitized lung tissue was preincubated at 37° for 15 min with various concentrations of TMQ (●), Iso (○) or DSCG (×) and challenged by addition of the antigen as described in the text.

Effects of TMQ, Iso, and DSCG on the *in Vitro* Anaphylactic Release of Histamine

Sensitized lung tissue was preincubated with various concentrations of TMQ, Iso or the antiallergic drug DSCG, and challenged by addition of the antigen. Histamine release was inhibited by the β-stimulants at concentrations higher than 10^{-10} M and by DSCG at higher than 10^{-7} M (Fig. 2). The two β-stimulants showed similar dose-response relationships except at very high concentrations.

In order to examine the effects of β-adrenergic blockers on the inhibition of histamine release by TMQ, sensitized lung tissue was preincubated with TMQ in the presence or absence of β-adrenergic blockers. As shown in Fig. 3 A, 10^{-5} M propranolol reversed the inhibition of histamine release by TMQ at concentrations lower than 10^{-5} M. In contrast, practolol did no modify the inhibitory activity of TMQ even at an antagonist-to-agonist ratio of 10^5 (Fig. 3 B).

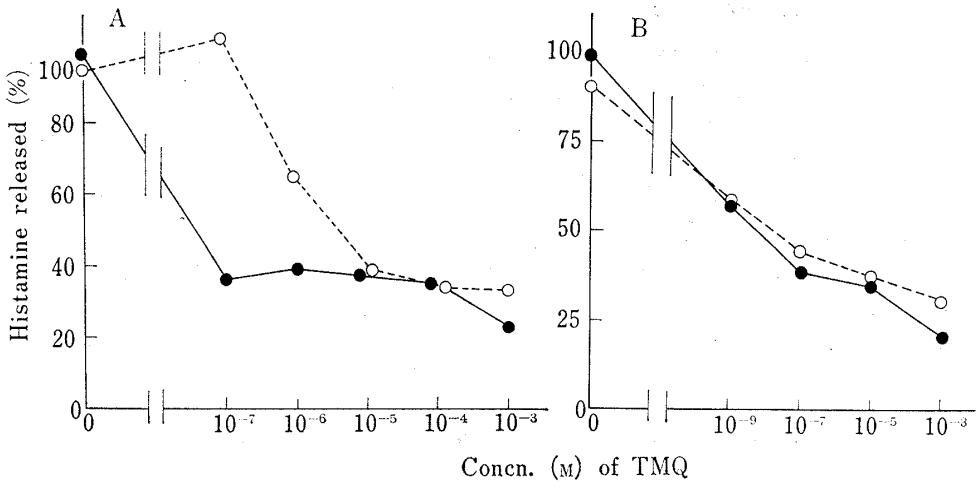

Fig. 3. Effects of Propranolol and Practolol on the Inhibition of Histamine Release by TMQ

Sensitized lung tissue was preincubated with TMQ at 37° for 15 min in the presence (○) or absence (●) of 10^{-5} M propranolol (A) or 10^{-4} M practolol (B), and then challenged as described in the text.

Effects of TMQ, Iso and DSCG on Anaphylactic Histamine Release from actively Sensitized Guinea Pig Lungs

Lungs of guinea pigs immunized actively with OA were minced, preincubated with test compounds and then challenged by addition of OA. Relative amounts (per cent of control tubes) of histamine released in the presence of 10^{-5} M and 10^{-3} M TMQ, Iso and DSCG were 69.0, 72.2, 97.1 and 36.0, 63.9, 64.9, respectively, TMQ being the most active of the three in this system.

Effects of TMQ on Cyclic AMP Levels of Rat Mast Cells

When the mast cell-rich fraction of rat peritoneal cells was incubated at 37° with 10^{-5} M or 10^{-3} M of TMQ or Iso, the β-stimulants elevated the cellular cAMP content, and this elevation was inhibited by prior incubation with propranolol, but not with practolol (Fig. 4).

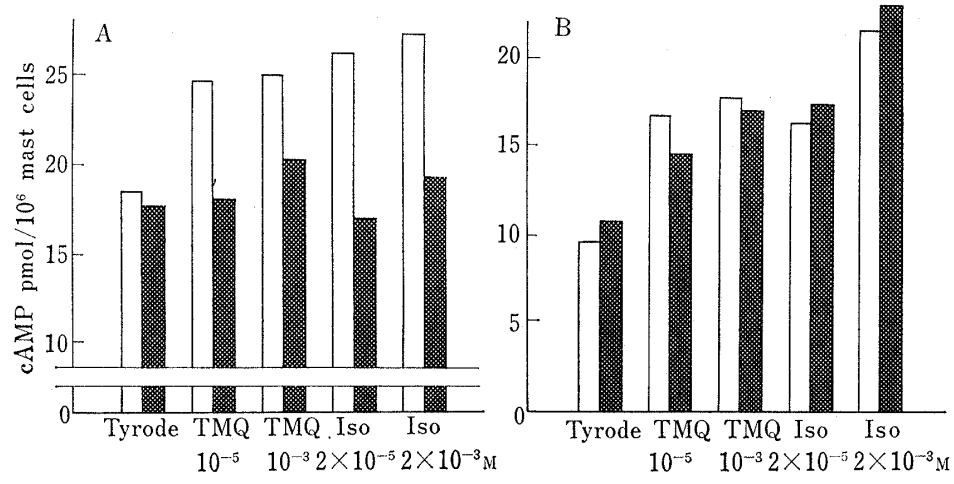

Fig. 4. Effects of TMQ and Iso on Intracellular cAMP of Rat Mast Cells

The mast cell-rich fraction of rat peritoneal cells was incubated at 37° first with 10^{-4} M propranolol (A) or practolol (B) for 2 min in the presence of 5×10^{-3} M theophylline, and then with TMQ or Iso for 1 min. Intracellular contents of cAMP were measured by radioimmunoassay as described in the text.
□: agonist+Tyrode, ▨: agonist+antagonist

Effects of TMQ, Iso and DSCG on Rat PCA

When sensitized rats were treated *i.v.* with TMQ or Iso at the time of challenge (*i.e.*, the drugs were added to the antigen solution), PCA reaction was inhibited by minute doses of these drugs (Table I, experiment 1). TMQ was 10 times more effective than Iso (ID_{100}) in this system. The efficacy of Iso in this experiment was comparable to those reported by other investigators.[10a,b,d] In order to examine the efficacy by the oral route, 1 mg/kg of TMQ or 2 mg/kg of Iso was administered *p.o.* to sensitized rats at various times before the challenge. The data showed different time courses of inhibition by the two β-stimulants (Fig. 5). Iso exhibited a maximal effect 5 min after administration, followed by a relatively rapid decrease in the activity. In contrast, TMQ showed relatively constant effects over the period of 5 to 30 min after administration, and the activity was observable for at least

Table I. Effects of TMQ, Iso and DSCG on Rat PCA

Experiment	Compound	Route of administration	Interval (min)	PCA response[a]				
1	Control			0.70±0.07				
				0.1	1	10	100	1000 μg/kg
	TMQ	*i.v.*	—	0.52±0.13	0±0[b]	0±0[b]	0±0[b]	0±0[b]
	Iso	*i.v.*	—		0.47±0.46	0±0[b]	0±0[b]	0±0[b]
2	Control			1.33±0.08				
				1	2 mg/kg			
	TMQ	*p.o.*	15	0.47±0.18[d]				
	Iso	*p.o.*	15		0.67±0.30[c]			
	Orciprenaline	*p.o.*	15		0.62±0.11[d]			
	Salbutamol	*p.o.*	15		1.09±0.19			
	Terbutaline	*p.o.*	15		0.68±0.39			
	DSCG	*p.o.*	15	1.15±0.12				

Rats were sensitized by injecting *i.c.* on the back 0.05 ml of 40 fold diluted rat anti-As antiserum, and 24 hr later challenged by injecting *i.v.* a solution containing the antigen and the dye as described in the text. The drugs were given *i.v.* along with the antigen or administered orally 15 min before the challenge.
a) The product of the largest diameter (cm) and its perpendicular diameter (cm) of the blueing area.
b) $p<0.05$.
c) $p<0.10$.
d) $p<0.01$.
Mean value with standard error (S.E.) ($n=5$).

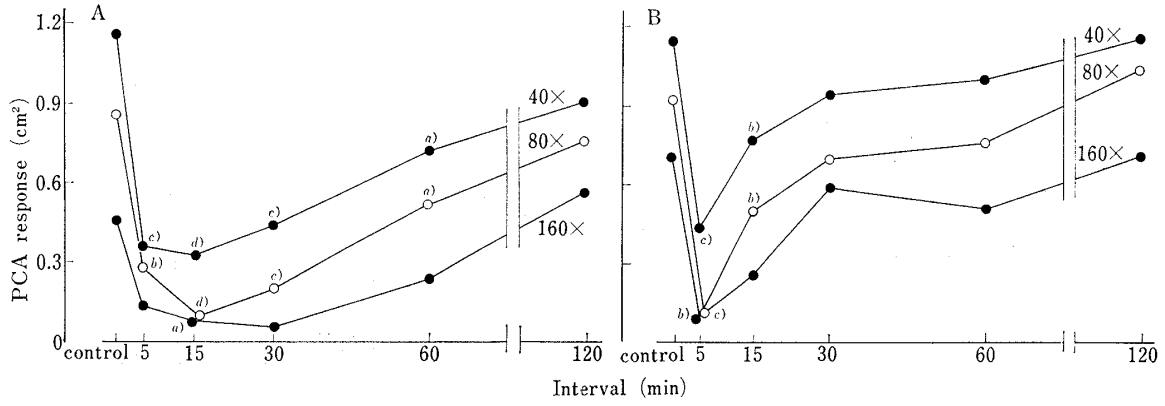

Fig. 5. Interval-response Relationship in the Inhibition of Rat PCA by TMQ and Iso

Sensitized rats were treated *p.o.* with 1 mg/kg of TMQ (A) or 2 mg/kg of Iso (B) at various times before the challenge. See the legend to Table I for the conditions of sensitization and challenge. Three dilutions (40 ×, 80 ×, and 160 ×) of the antiserum were used.
$n=5$. *a*) $p<0.10$; *b*) $p<0.05$; *c*) $p<0.01$; *d*) $p<0.001$.

60 min. When the *p.o.* effects of TMQ (*l*-isomer, 1 mg/kg), other β-stimulants (racemic compounds of Iso, orciprenaline, salbutamol and terbutaline, 2 mg/kg) and DSCG (2 mg/kg of a 50% preparation) were compared 15 min after administration, TMQ was the most effective (Table I, experiment 2).

Effects of TMQ, Iso and DSCG on Guinea Pig PCA

The effects of TMQ, Iso or DSCG on guinea pig PCA were investigated by sensitization with both guinea pig and rabbit antisera. The drugs were administered *i.p.* 15 min before the challenge. Ten mg of TMQ per animal inhibited both homologous and heterologous PCA by 35.1 and 35.6%, respectively ($p<0.01$). Neither 10 mg of Iso nor 10 and 20 mg of DSCG showed a significant inhibition. After administration of 20 mg of Iso, all the guinea pigs (whether sensitized or unsensitized) died in a few minutes.

Effects of TMQ and Iso on Systemic Anaphylaxis in Guinea Pigs

Guinea pigs were passively sensitized by injecting *i.v.* guinea pig anti-OA antiserum and challenged by injecting *i.v.* a solution containing the antigen and test drugs. As shown in Table II, TMQ (1.9 mg per animal) was more effective than Iso (5.0 mg) in inhibiting the systemic anaphylactic reactions. Judging from the ratios of incidence of the anaphylactic responses, 0.9 mg of TMQ was equivalent to 5 mg of Iso.

Table II. Effects of TMQ and Iso on Systemic Anaphylaxis in Guinea Pigs

Compound	Dose	Onset time of anaphylactic responses (min)			Incidence rate	
		Convulsion	Spasmodic inspiration	Termination of breathing	Anaphylaxis	Death
Control	—	2	2	—	4/4	3/4
		1	2	4		
		1	2	4		
		1	1	4		
TMQ	0.9 mg	—	—	—	3/5	2/5
		—	—	—		
		5	—	—		
		1	5	7		
		1	2	5		
	1.9	—	—	—	0/5	0/5
		—	—	—		
		—	—	—		
		—	—	—		
		—	—	—		
	3.8	—	—	—	1/5	1/5
		—	—	—		
		—	—	—		
		—	—	—		
		2	2	4		
Iso	5.0	—	—	—	3/5	1/5
		—	—	—		
		3	4	—		
		2	3	—		
		1	2	5		

Guinea pigs were passively sensitized by injecting *i.v.* 1 ml of 25 fold diluted guinea pig anti-OA antiserum, and 24 hr later challenged by injecting *i.v.* a solution containing the test compound and 0.03 mg of OA. Times between the challenge and the onset of anaphylactic responses in each animal were recorded in minutes.

Effects of TMQ and Iso on Histamine-induced Dye Leakage

In order to examine the effects of orally administered TMQ and Iso on vascular permeability, histamine was injected *i.c.* on the backs of rats which had been treated with test

compounds and Evans blue, and the size of the resulting blue spots was measured (Fig. 6). Iso inhibited dye leakage markedly, with maximal effects at 5 min after administration, whereas TMQ had only slight effects on vascular permeability.

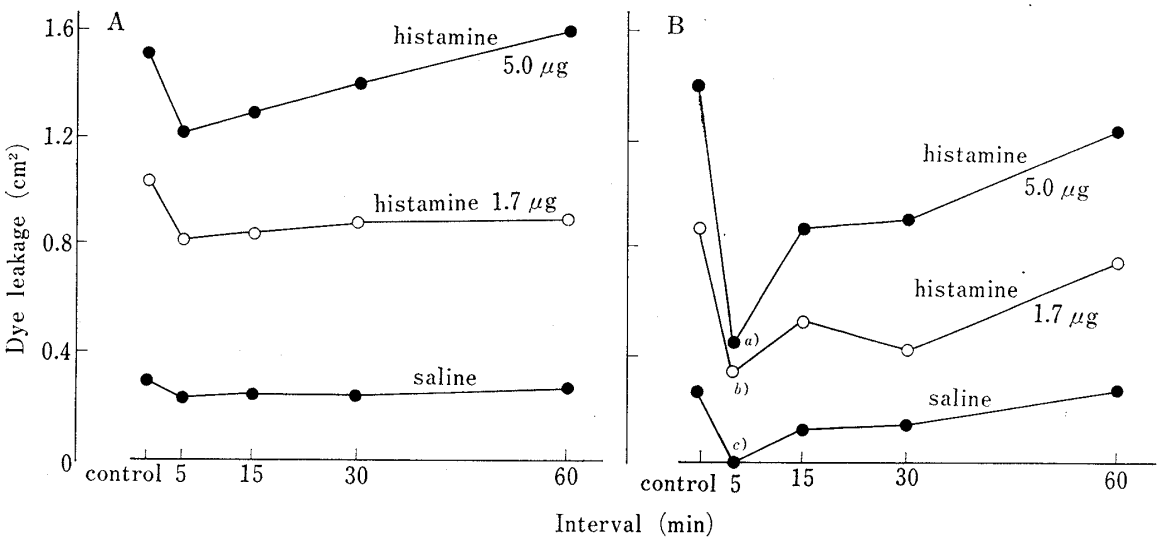

Fig. 6. Interval-response Relationship in the Effects of TMQ and Iso on Histamine-induced Dye Leakage

Rats were treated *p.o.* with 1 mg/kg of TMQ (A) or Iso (B) at various times, and were injected *i.v.* with Evans blue 10 min before the *i.c.* injection of histamine hydrochloride. $n=3$. *a*) $p<0.05$; *b*) $p<0.01$; *c*) $p<0.001$.

Discussion

Histamine release from the minced guinea pig lung tissue sensitized *in vitro* with heterologous (rabbit) antiserum was immunologically specific and dependent on the antigen dose (Fig. 1 A), affording a convenient system for studying drug effects on anaphylactic histamine release. Under the conditions used, maximal release occurred on challenging with 10 or 100 μg/ml of antigen, and concentrations higher than that caused a reduction of the release. This phenomenon, also observed (but not commented on) by Ishizaka *et al.*,[18] could be explained as follows: when the number of the antigen molecules added is greater than that of the antibodies fixed on the mast cell surface, one antigen molecule may combine with only one antibody molecule, and hence the cross-linkage among the antigen-antibody complexes on the cell surface, which is presumably necessary for the release of allergic mediators,[19] will not occur. Excessive concentrations of antibody also diminish the subsequent histamine release, possibly by preventing the effective binding of the antigen on the cell surface which is overcrowded with antibody molecules (Fig. 1 B).

TMQ and Iso inhibited the anaphylactic reactions both *in vitro* and *in vivo* more effectively than DSCG. Although the *in vitro* potencies of the two β-adrenomimetic agents in inhibiting histamine release (Fig. 2) and elevating intracellular levels of cAMP in mast cells (Fig. 4) did not differ greatly, TMQ appeared to be more active *in vivo* than Iso in its effects on both rat PCA (Table I, experiment 2) and systemic anaphylaxis in guinea pigs (Table II).

The inhibition of PCA could arise by two mechanisms, *i.e.*, (1) inhibition of mediator release, and (2) inhibition of dye leakage (decreased vascular permeability). It has recently

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been reported that β-stimulants modified vascular injury and inhibited dye leakage.[20] In the present study, TMQ showed no significant effect on histamine-induced dye leakage, but Iso markedly inhibited it with maximal effects at 5 min after administration (Fig. 6). Thus, a substantial part of the inhibition of PCA by Iso at 5 min (Fig. 5 B) may be ascribed to the inhibition of vascular permeability by this compound. Consequently, Iso is likely to be less potent as an *in vivo* inhibitor of the anaphylactic mediator release than it appears from the PCA inhibition data (Fig. 5 B), and this may also explain the higher activity of TMQ compared to Iso in inhibiting systemic anaphylaxis (Table II).

The inhibition of histamine release *in vitro* and the elevation of cAMP levels in mast cells by TMQ were both antagonized by the nonspecific β-blocker propranolol, but not by the β_1-specific blocker practolol. These results could be taken as evidence that β_2-adrenergic receptors are responsible for both the inhibition of anaphylactic histamine release and the elevation of mast cell cAMP by TMQ. As adrenergic β-stimulants, both TMQ and Iso cause relaxation of tracheal muscle (β_2-receptor) and stimulate the heart (β_1-receptor); TMQ is more potent than Iso with respect to the former action whereas the relation is reversed for the latter.[11b] Therefore, the greater anti-anaphylactic activity of TMQ compared with Iso suggests that mast cells or other cells involved in anaphylactic release may carry β_2-receptors. There seems to be some confusion in the literature concerning the β-adrenoreceptor of guinea pig lung mast cells. Assem and Schild[3b] suggested that both β_1- and β_2-receptors may be involved in the inhibition of anaphylactic histamine release by Iso. Malta and Raper[21] concluded that β-receptors mediating the above inhibition differed from those found in atria (β_1) and trachea (β_2) of the guinea pig. Sörenby,[22] however, showed in his *in vitro* anaphylactic histamine release study that the receptors concerned are more related to those mediating trachea relaxation than to those mediating cardiac stimulation.

In time-course studies the early onset of the TMQ effect is in line with the early absorption from the gut and fast distribution to the skin of orally administered TMQ in mice as observed in whole-body autoradiographic studies by Otsuka *et al.*,[23] and also with the prompt suppression of rat PCA by *i.v.* injection of as little as 0.1 μg/kg of TMQ shown in the present study (Table I, experiment 1). It is also noteworthy that TMQ and Iso showed different time courses of the inhibitory effect on rat PCA after oral administration (Fig. 5). In contrast to TMQ, the inhibitory effect of orally administered Iso on PCA or on vascular permeability was maximal at 5 min and decreased rapidly thereafter (Fig. 5 B and Fig. 6 B). This rapid recovery from the inhibition by Iso could be explained by three possibilities: (1) the interval-response curves of Iso may reflect the pattern of blood levels of this drug, which is determined by the rates of intestinal absorption, metabolic inactivation, and excretion, (2) above a critical blood concentration, Iso or its metabolite (s) may produce an opposite effect, *i.e.*, enhancement of vascular permeability counteracting the initial inhibitory effects, and (3) accumulation in blood of the metabolite (s) might block the binding of Iso to its receptors. These mechanisms would also contribute to the weaker *in vivo* effects of Iso on PCA (Table I) and systemic anaphylaxis (Table II) than expected from its *in vitro* effects on histamine release (Fig. 2) and intracellular levels of cAMP in mast cells (Fig. 4). Portmann *et al.*[24] and Conway *et al.*[25] investigated the absorption of orally administered Iso in dogs by following the time course of its cardiac effect. Their data also showed a prompt increase in heart rate (maximum within 5 min) and a relatively rapid decay of the pharmacological effect. The cardiac effect of intravenously administered Iso declined with a half-life of *ca.* 1 min. According to Conway

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et al.,[25] a large portion of orally administered Iso is inactivated by conversion to a sulfuric acid ester either by the intestinal flora or during passage through the intestinal wall, and the rest is largely inactivated by conversion to the 3-O-methyl derivative on passage through the liver. Thus, the quick metabolic inactivation may not allow free Iso to accumulate in the blood even if the intestinal absorption continues. In this case it is probable that the blood concentration of Iso is directly related to the rate of absorption, which is in turn a function of the concentration of Iso at the absorption site within the gastrointestinal tract, and is expected to reach a maximum shortly after oral administration. The possibility that higher blood concentrations of Iso might have increased vascular permeability has been excluded by our observation that intravenous administration of 1, 10, 100 and 1000 μg/kg of Iso produced dose-dependent inhibitions of histamine-induced dye leakage in rats (data not shown). However, the possibility that one of the Iso metabolites may exert a counteracting effect either directly on the capillary or as a β-adrenergic antagonist remains to be explored. Philippot *et al.*[26] and Paterson[27] reported that 3-O-methyl Iso was a β-adrenergic antagonist in cat and man.

The efficacy of β-adrenergic stimulants in rat PCA at intravenous doses comparable to those used clinically for human asthma strongly suggests that they may relieve allergic asthma not only by relaxation of the bronchial muscle but also by inhibiting the anaphylactic release of histamine and other mediators from the mast cells. As a therapeutic agent, TMQ may have an advantage over Iso in that TMQ may show a longer duration of action than Iso when administered orally in humans.

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