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Inhibitory Effect of HSR-6071, A New Anti-allergic Agent, on Experimental Asthma in Rats and Guinea-pigs

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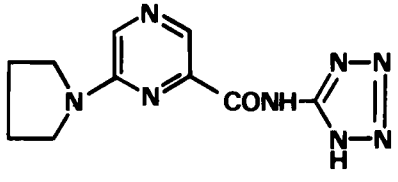
Abstract—The experimental asthma caused by IgE antibody in rats was inhibited by HSR-6071 (6-(1-pyrrolidinyl)-*N*-(1H-tetrazol-5-yl)-2-pyrazinecarboxamide) (0·01–0·1 mg kg^{-1} i.v.) in a dose-dependent manner. The inhibitory activity of HSR-6071 was more potent than those of disodium cromoglycate and ketotifen, and equipotent with amlexanox. The bronchoconstriction mediated by IgE or IgG antibody in guinea-pigs was also prevented by HSR-6071 (0·3, 1 and 3 mg kg^{-1} i.v.), amlexanox (3, 10 and 30 mg kg^{-1} i.v.) and ketotifen (0·1 mg kg^{-1} i.v.) but not by disodium cromoglycate (10 mg kg^{-1} i.v.). HSR-6071 was more potent than amlexanox, but less potent than ketotifen. HSR-6071 suppressed antigen-induced histamine and SRS-A release from minced lung tissues of guinea-pigs sensitized passively with rabbit anti-EA serum and was a more potent inhibitor of the release of SRS-A than of histamine. On the other hand, histamine- or acetylcholine-induced bronchoconstriction in guinea-pigs was scarcely affected by HSR-6071 at doses sufficient to inhibit the experimental asthma, but LTD_4-induced bronchoconstriction was dramatically inhibited. These results indicate that the inhibitory action on experimental allergic asthma of HSR-6071 may be due to suppression of antigen-induced histamine and SRS-A release from lung tissues and to antagonism of SRS-A action. In addition, HSR-6071 inhibited cyclic AMP phosphodiesterase activity and produced relaxation of the guinea-pig isolated trachea. These pharmacological actions may contribute to the production of the anti-allergic action of HSR-6071.

We have reported (Makino et al 1989) that HSR-6071, a novel pyrazinecarboxamide derivative, exhibits potent inhibition of the IgE-mediated passive cutaneous anaphylaxis (PCA) in rats on oral administration, and that its inhibition of PCA is at least in part due to the depression of allergic histamine release from mast cells. The present experiments were designed to investigate the effect of HSR-6071 (6-(1-pyrrolidinyl)-*N*-(1H-tetrazol-5-yl)-2-pyrazinecarbox-amide) on experimental allergic asthma in rats and guinea-pigs compared with those of disodium cromoglycate, amlexanox and ketotifen and to analyse the mechanism of the action.



Materials and Methods

Animals

Hartley guinea-pigs of either sex (300–600 g), male Wistar rats (200–220 g), and male Japanese White rabbits (2–2·5 kg) were purchased from Japan SLC Inc. (Hamamatsu, Japan). They were housed in an air-conditioned room at 24±2°C and fed the usual laboratory diet with free access to water.

Chemicals

The chemicals used were: histamine dihydrochloride (Nacalai Tesque), acetylcholine chloride (Daiichi), leukotriene D_4 (Funakoshi), bovine serum albumin (BSA), egg albumin (EA), isoprenaline hydrochloride and potassium benzylpenicillin (all from Sigma), bovine gamma globulin (Miles), sodium 2,4-dinitrophenyl sulphate (Tokyo Kasei), Adjuvant *Bordetella pertussis* (Kaken Seiyaku), Freund's complete adjuvant (DIFCO), disodium cromoglycate (DSCG; Star), ketotifen fumarate (Resfar), amlexanox (Takeda), theophylline (Wako), propranolol hydrochloride (Sumitomo Seiyaku), FPL-55712 and HSR-6071 (synthesized in our laboratory). HSR-6071 and amlexanox were dissolved in 0·1 M NaOH and neutralized with 0·1 M HCl and diluted with 0·9% NaCl (saline) for i.v. administration or with distilled water for in-vitro studies to concentrations required. DSCG, ketotifen and theophylline were dissolved in saline or distilled water.

Antisera

Rat anti-dinitrophenylated ascaris-extract (DNP-As) serum containing IgE antibody was prepared by the method of Tada & Okumura (1971). The PCA titre of the antiserum was 1:128 as measured by 48-h PCA in rats. Guinea-pig anti-benzylpenicilloyl bovine gamma globulin (BPO-BGG) serum containing IgE antibody was prepared by the method of Levine et al (1971). The PCA titre of the antiserum was 1:1024 as measured by 7-day PCA in guinea-pigs. Rabbit anti-egg albumin (EA) serum containing IgG antibody was prepared by the method of Koda et al (1970). The 4 h PCA titre in guinea-pigs was 1:16,384.

Experimental allergic asthma in rats

Induction and evaluation of the asthmatic symptoms were

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according to the method of Koda et al (1978). Allergic asthma was induced in passively sensitized rats with 5 mL kg^{-1} i.v. of the rat anti-DNP-As serum. Twenty four h after the sensitization, tracheotomy was performed under urethane anaesthesia (1·1 g kg^{-1} i.p.) and the asthmatic response induced by challenge with 12 mg kg^{-1} of DNP-As as an antigen. The rate and volume of respiration were measured by respirometer (air flow resistance tube: TV-241T, pressure transducer: TP-602T, temperature control box: RY-111S, respiratory amplifier: AR-601G, respiratory volume unit: AQ-601G, Nihon Koden). Both parameters were measured at 0·5, 1, 2, 3, 4, 5, 7, 10 and 15 min after the antigen challenge. The rate and volume of respiration and the ratio of expiratory time vs inspiratory time were calculated from the respiratory curves and each parameter was expressed as a percentage of the corresponding value before the antigen challenge. Test compounds were given i.v. 1 min before the antigen challenge.

Experimental allergic asthma in guinea-pigs

IgE-mediated bronchoconstriction was induced in guinea-pigs sensitized passively with 0·8 mL kg^{-1} i.v. of guinea-pig anti-BPO-BGG serum. Tracheotomy was performed under urethane anaesthesia (1·5 g kg^{-1} i.p.) 48 h after the sensitization. Airway resistance was measured according to the technique of Konzett & Rössler (1940) with a minor modification. An increase in respiratory overflow volume induced by the challenge of antigen (BPO–BSA, 50 μg kg^{-1} i.v.) was expressed as a percentage of the maximal overflow volume (100%) which had been obtained by occluding the trachea. Test compounds were given i.v. 1 min before the antigen challenge.

Heterologous IgG-mediated bronchoconstriction was induced in guinea-pigs sensitized passively with 0·2 mL kg^{-1} i.v. of rabbit anti-EA serum. Twenty four h after the sensitization, the airway resistance was measured by the same procedure as described above, except for the amount of antigen (500 μg kg^{-1}).

LTD_4-, histamine- and acetylcholine-induced bronchoconstrictions in guinea-pigs

The bronchoconstriction induced by histamine (5 μg kg^{-1} i.v.), acetylcholine (40 μg kg^{-1} i.v.) or LTD_4 (0·2 μg kg^{-1} i.v.) was measured as described above.

Immunological histamine and SRS-A release from passively sensitized guinea-pig lung fragments

Guinea-pigs were passively sensitized with 0·1 mL kg^{-1} of rabbit anti-EA serum. The lungs were removed 24 h after the sensitization and minced into 2–3 mm fragments. Minced tissue (300 mg) was suspended in 2·7 mL of Tyrode solution and prewarmed at 37°C for 10 min. Then, the suspension was incubated in the presence of EA in a final concentration of 100 μg mL^{-1} at 37°C for 20 min. Test compounds or vehicle were added 5 min before the antigen challenge. The amount of histamine in the supernatant was measured by the fluorometric method of Shore et al (1959). SRS-A was determined by bioassay using guinea-pig ileum in the presence of atropine (3×10^{-8} M) and mepyramine (3×10^{-7} M). The change in tension was recorded on a pen-writing oscillograph (R-64, Rikadenki) through an isotonic transducer (TD-112S, Nihon Koden or ME-4012, MEC). The amount of SRS-A referred to a contraction of guinea-pig ileum equal to that induced by 5 ng histamine according to the method of Saijo et al (1985).

Measurement of cyclic (c) AMP phosphodiesterase (PDE) activity

Guinea-pig isolated lung was chopped and homogenized in ice-cooled 50 mM Tris-HCl buffer containing 5 mM $MgCl_2$ (pH 8·0). The supernatant was obtained by centrifugation at 1300 *g* for 10 min, and further centrifuged at 22000 *g* for 30 min to obtain the crude AMP PDE (1·91 mg protein mL^{-1}). The activity of this enzyme was measured according to Russell et al (1972). Briefly, the enzyme solution was diluted 5-fold with 50 mM Tris-HCl buffer supplemented with 5 mM $MgCl_2$ and 3·75 mM 2-mercaptoethanol (Wako). The reaction mixture in the presence or absence of test compounds was incubated for 10 min at 30°C. After addition of [^{3}H] cAMP (NEN) and cold cAMP (Seikagaku Kogyo), the reaction mixture was allowed to incubate for the following 15 min. The reaction was terminated by boiling for 3 min, and the mixture was then incubated in the presence of king cobra venom (Sigma) at a final concentration of 250 μg mL^{-1} at 30°C for 10 min. One mL of a 50% suspension of Dowex 1-X2 (Dow Chemical) was added to remove unconverted cAMP and followed by centrifugation at 700 *g* for 15 min. [^{3}H]Adenosine as a final product was determined by a liquid scintillation counter (LSC-1000, Aloka).

Relaxing activity in guinea-pig isolated trachea

Guinea-pigs anaesthetized with ethyl ether were killed by stunning and the trachea was rapidly excised. A chain strip of trachea was suspended vertically under a 0·5 g load in an organ bath containing 10 mL of Locke-Ringer solution maintained at 37°C and bubbled with a gas mixture of 95% O_2 and 5% CO_2. Changes in isotonic tension were recorded by means of a force-displacement transducer (TD-112S, Nihon Koden or MEC-4021, MEC). The ability of compounds to relax smooth muscle was determined on the quiescent, histamine(10^{-5} M)- and LTD_4(10^{-8} M)-contracted guinea-pig trachea. A cumulative concentration-response curve was generated for each compound and finally, 10^{-6} M of isoprenaline was added into the organ chamber. The percent relaxation was calculated by dividing the relaxation produced by the various concentrations of test compounds by that induced by 10^{-6} M isoprenaline.

Statistical analysis

The values obtained were expressed as the mean ± s.e. Student's *t*-test was used to evaluate the statistical significance between two means.

Results

Effect on experimental allergic asthma in rats

A typical pattern of the antigen-induced asthmatic respiration in rats is shown in Fig. 1. A decrease in respiratory rates and volume, and an extension of expiratory time were caused immediately after the antigen challenge. The maximum responses were observed at 1–2 min after the antigen challenge and gradually returned to the control level with the lapse of time.

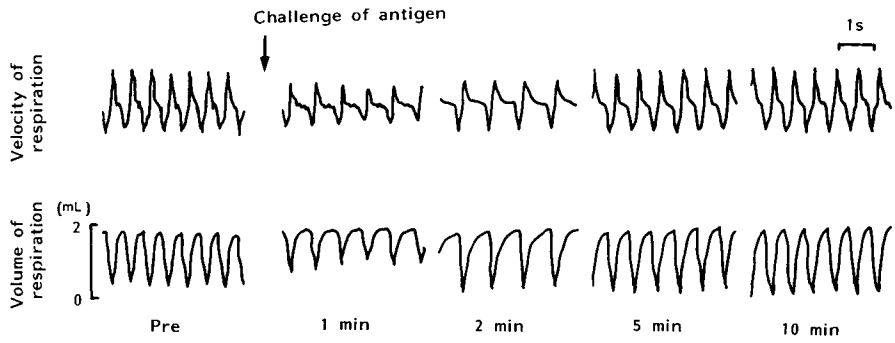

FIG. 1. Representative asthmatic response in rats. Changes in respiratory rate and volume were determined in the rat sensitized passively with anti-DNP-As serum. Asthmatic response was induced by the challenge with DNP-As 24 h after the sensitization.

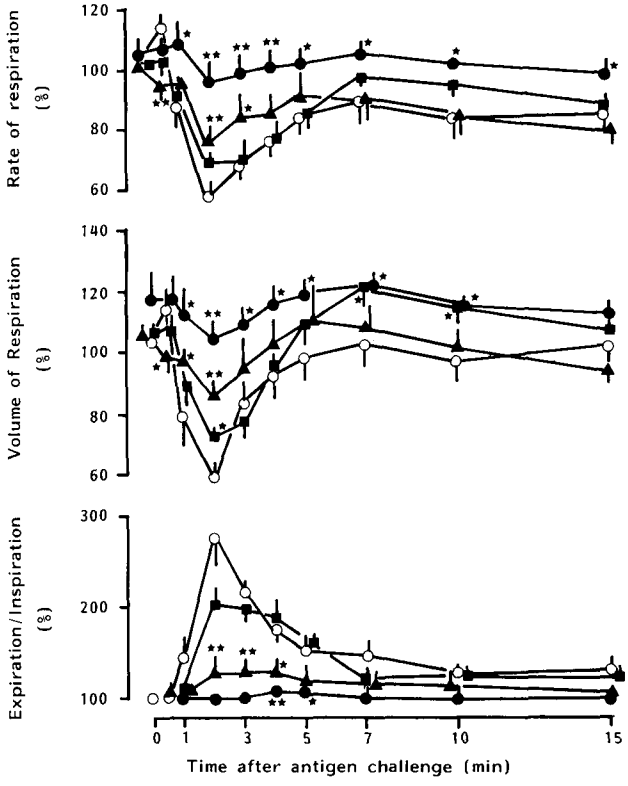

FIG. 2. Effect of HSR-6071 on experimental asthma in rats. Rats were passively sensitized with anti-DNP-As serum and challenged with DNP-As. HSR-6071 was given i.v. 1 min before the antigen challenge. Each point represents the mean ± s.e. of 7 to 8 animals. (-○-): Control (saline), (-■-): 0·01 mg kg^{-1}, (-▲-): 0·03 mg kg^{-1}, (-●-): 0·1 mg kg^{-1}. * $P<0·05$, ** $P<0·01$ compared with the corresponding control.

As shown in Fig. 2, HSR-6071 (0·01, 0·03 and 0·1 mg kg^{-1} i.v.) prevented the asthmatic respiratory response in a dose-dependent fashion. The effect of HSR-6071, amlexanox, DSCG and ketotifen on the experimental asthma is summarized as shown in Table 1. HSR-6071 was more potent than DSCG and ketotifen, and nearly equipotent with amlexanox in this model.

Effect on the experimental allergic asthma in guinea-pigs

As shown in Fig. 3a, an increase in respiratory overflow resulting from IgE-mediated bronchoconstriction in guinea-pigs reached a peak of 70·8 ± 9·0% 4 min after the antigen (BPO-BSA) challenge and then recovered slowly to the normal level. The per cent bronchoconstriction at 15 min after the antigen challenge was 32·7 ± 9·5%. When given 1 min before the antigen challenge, HSR-6071 in doses of 1 and 3 mg kg^{-1} i.v. suppressed the bronchoconstriction by 10·7 and 69·4%, respectively. Amlexanox (30 mg kg^{-1} i.v.) and ketotifen (0·1 mg kg^{-1} i.v.) significantly ($P<0·05$ or $P<0·01$) inhibited the bronchoconstriction, but DSCG (10 mg kg^{-1} i.v.) had no effect (Table 2).

In heterologous IgG-mediated bronchoconstriction, the peak response (89·6 ± 4·2%) was observed 2 min after the antigen challenge and recovered rather slower than that seen in the case of IgE-mediated bronchoconstriction (Fig. 3b). HSR-6071 in doses of 0·3 and 1 mg kg^{-1} i.v. inhibited the bronchoconstriction by 41·6 and 60·9%, respectively. Amlexanox (10 mg kg^{-1} i.v.) and ketotifen (0·1 mg kg^{-1} i.v.) significantly ($P<0·05$ or $P<0·01$) inhibited the bronchoconstriction, while DSCG (10 mg kg^{-1} i.v.) was without effect (Table 2).

Table 1. Effect of HSR-6071, amlexanox, DSCG and ketotifen on experimental asthma in rats.

Compound	Dose (mg kg^{-1})	% inhibition of asthmatic respiration: Rate	Volume
HSR-6071	0·01	20·3 ± 7·5	21·6 ± 6·6
	0·03	33·1 ± 8·4*	46·1 ± 10·6*
	0·1	65·3 ± 11·8**	78·1 ± 11·4**
Amlexanox	0·01	21·4 ± 17·8	33·9 ± 25·2
	0·03	45·2 ± 18·3	58·1 ± 19·9*
	0·1	64·2 ± 6·6**	86·7 ± 6·9**
DSCG	0·1	15·8 ± 5·8	21·2 ± 9·8
	0·3	41·2 ± 19·2	73·7 ± 24·6*
	1·0	67·4 ± 6·5**	87·2 ± 11·6**
Ketotifen	0·1	7·6 ± 11·8	7·3 ± 14·4
	0·3	52·0 ± 7·8**	54·4 ± 11·8**
	1·0	78·2 ± 15·3**	105·7 ± 28·5**

Asthmatic response was induced in rats sensitized passively with rat anti-DNP-As serum and estimated as the changes in respiratory rate and volume (see text). Each compound was given i.v. 1 min before the antigen challenge. Each value represents the mean ± s.e. of the percent inhibition vs control at the peak time. Each group consisted of 7 or 8 animals. * $P<0·05$, ** $P<0·01$, compared with the control.

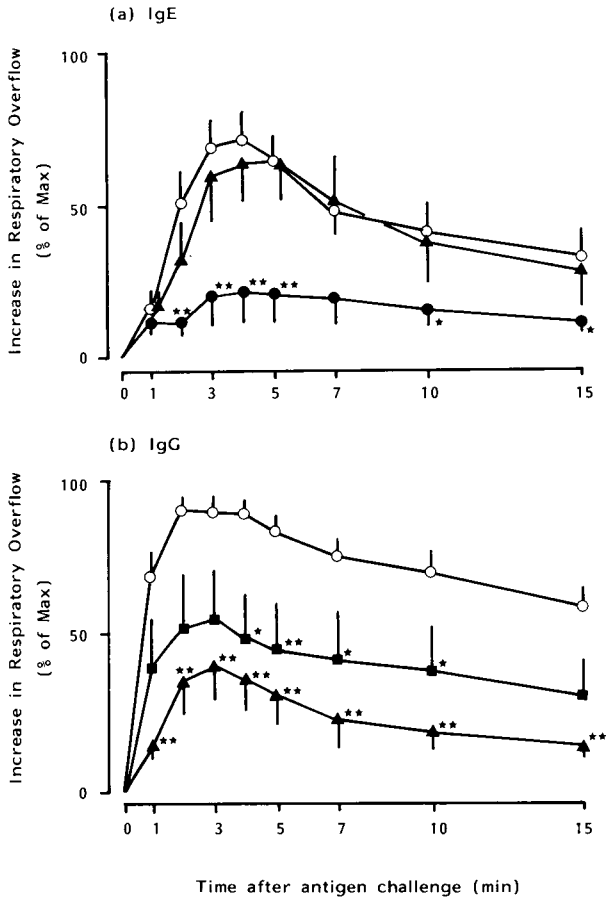

FIG. 3. Effect of HSR-6071 on the IgE (a)- and IgG (b)-mediated bronchoconstriction in guinea-pigs. HSR-6071 was given i.v. 1 min before the antigen challenge. Each point represents the mean ± s.e. of 7 to 22 animals. (-○-): Control (saline), (-■-): 0·3 mg kg^{-1}, (-▲-): 1 mg kg^{-1}, (-●-): 3 mg kg^{-1}, * $P<0·05$, ** $P<0·01$ compared with corresponding control.

Table 2. Effect of HSR-6071, amlexanox, DSCG and ketotifen on the IgE (a)- and IgG (b)-mediated bronchoconstriction in guinea-pigs.

Exp.	Compound	Dose (mg kg^{-1})	n	% inhibition of bronchoconstriction
a	HSR-6071	1	8	10·7 ± 19·1
		3	8	69·4 ± 15·5**
	Amlexanox	10	9	12·3 ± 16·2
		30	8	56·2 ± 16·2*
	DSCG	10	8	28·5 ± 21·2
	Ketotifen	0·1	8	90·7 ± 2·0**
b	HSR-6071	0·3	7	41·6 ± 19·3
		1	7	60·9 ± 12·4**
	Amlexanox	3	7	44·2 ± 20·1
		10	7	58·1 ± 18·6*
	DSCG	10	7	13·2 ± 12·5
	Ketotifen	0·1	7	88·6 ± 4·2**

The bronchoconstriction was induced in guinea-pigs sensitized passively with guinea-pig anti-BPO-BGG serum or rabbit anti-EA serum (see text). Each compound was given i.v. 1 min before the antigen challenge. Each value represents the mean ± s.e. of the per cent inhibition vs control at the peak time. Control groups in a and b consisted of 17 and 22, respectively. Others consisted of 7 to 9 animals. * $P<0·05$, ** $P<0·01$ compared with the control.

In these two models, ketotifen was the most potent among the agents tested. The inhibitory effect of HSR-6071 was about 10 times more potent than that of amlexanox.

Effect on LTD_4-, histamine- and acetylcholine-induced bronchoconstrictions in guinea-pigs

LTD_4 in doses of 0·1 to 0·4 μg kg^{-1} i.v. provoked a dose-dependent bronchoconstriction. To examine the effect of the test agents, 0·2 μg kg^{-1} i.v. of LTD_4, which induced 73·1 ± 5·5% increase in the respiratory overflow, was used. When given 1 min before the administration of LTD_4, HSR-6071 in doses of 0·2 to 1 mg kg^{-1} i.v. caused a dose-dependent inhibition of the agonist-induced response, which was also inhibited by amlexanox at doses of 5 and 10 mg kg^{-1} i.v. but not by DSCG (10 mg kg^{-1} i.v.) and ketotifen (1 mg kg^{-1} i.v.) (Table 3). FPL-55712, an LTD_4 antagonist, exhibited the significant suppression at doses of 0·1 and 0·2 mg kg^{-1} i.v.

Histamine (5 μg kg^{-1} i.v.) and acetylcholine (40 μg kg^{-1} i.v.) elicited a transient bronchoconstriction in guinea-pigs with the peak response of 90·0 ± 5·6 and 60·0 ± 5·5%, respectively. HSR-6071 at doses of 1 and 3 mg kg^{-1} i.v. 1 min before the injection of histamine, or acetylcholine had little effect on the agonist-induced bronchoconstriction (data not shown). The histamine-induced bronchoconstriction was, however, inhibited by ketotifen (0·1 mg kg^{-1} i.v.) (data not shown).

Effect on immunological histamine and SRS-A release from guinea-pig lung tissues

Net histamine and SRS-A release resulting from the antigen (100 μg mL^{-1} of egg albumin) challenge were 6·47 ± 0·37 μg and 64·4 ± 5·43 units per g of lung, respectively. As shown in Table 4, HSR-6071 (10^{-5}–10^{-3} M) showed a concentration-dependent inhibition of histamine and SRS-A release and was a more potent inhibitor for SRS-A release than for histamine release. Amlexanox (10^{-5}–10^{-3} M) produced a concentration-dependent inhibition of the SRS-A release, but not the histamine release at concentrations of 10^{-5} and 10^{-4} M. DSCG (10^{-5} to 10^{-3} M) and ketotifen (10^{-6} and 10^{-5} M) did not inhibit either of the releases.

Effect on cAMP-PDE activity

The results obtained were analysed according to the method

Table 3. Effect of HSR-6071, amlexanox, DSCG, ketotifen and FPL55712 on the LTD_4-induced bronchoconstriction in guinea-pigs.

Exp.	Compound	Dose (mg kg^{-1})	n	bronchoconstriction (% of max)
1	Control	—	7	87·2 ± 3·3
	HSR-6071	0·2	7	66·7 ± 5·5**
		0·5	7	61·1 ± 9·6*
		1	7	28·6 ± 6·3**
	FPL55712	0·1	7	51·1 ± 8·0**
		0·2	7	11·3 ± 5·4**
	Ketotifen	1	7	65·4 ± 11·3
2	Control	—	7	77·2 ± 5·6
	Amlexanox	5	7	33·4 ± 8·4**
		10	7	23·8 ± 6·0**
	DSCG	10	7	72·4 ± 6·3

Each compound was given i.v. 1 min before the injection of LTD_4 (0·2 μg kg^{-1} i.v.). Each value represents the mean ± s.e. of 7 animals. * $P<0·05$, ** $P<0·01$, compared with the control.

Table 4. Effects of HSR-6071, amlexanox, DSCG and ketotifen on the immunological histamine and SRS-A release from passively sensitized guinea-pig lung tissues.

Compound	Concn (M)	n	% inhibition of the release: Histamine	SRS-A
HSR-6071	10^{-5}	3	7·2±7·0	21·3±14·5
	10^{-4}	3	26·8±6·4**	54·1±6·8**
	10^{-3}	3	45·7±5·4**	83·5±0·4**
Amlexanox	10^{-5}	3	7·6±8·1	35·5±8·2
	10^{-4}	3	5·4±2·1	54·0±5·0**
	10^{-3}	3	23·9±3·8*	94·5±0·4**
DSCG	10^{-4}	2	−14·7	−2·4
	10^{-3}	2	−9·9	−7·4
Ketotifen	10^{-6}	3	−16·5±7·5	−29·8±27·2
	10^{-5}	3	−24·5±7·8	−35·9±14·3

Passively sensitized lung tissues were preincubated with each compound 5 min before the antigen (egg albumin) challenge (see test). Net histamine and SRS-A release resulting from the antigen challenge were 6·57±0·37 μg and 64·4±5·43 units per g of lung, respectively. Each value represents mean±s.e. of 2 to 3 experiments. * $P<0.05$, ** $P<0.01$ compared with the control.

of Lineweaver & Burk (1934). As shown in Fig. 4, HSR-6071, as well as DSCG, inhibited cAMP-PDE. In contrast, the enzyme inhibition with theophylline was competitive. In this respect, the mode of inhibition appeared to be different between HSR-6071 and theophylline. The K_i values of HSR-6071, DSCG and theophylline were estimated to be 2.15×10^{-4}, 3.80×10^{-3} and 2.69×10^{-4} M, respectively.

Relaxing action in the isolated trachea

HSR-6071 was compared with theophylline for the ability to relax the isolated trachea from guinea-pigs. As shown in Fig. 5, both compounds produced a concentration-dependent relaxation in the quiescent state and during the contraction with histamine and LTD_4. The EC50 (concentration producing 50% relaxation) values for HSR-6071 and theophylline were 7.2×10^{-5} and 4.5×10^{-5} M in the quiescent state, 1.1×10^{-4} and 1.0×10^{-4} M during histamine-induced contraction and, 1.3×10^{-4} and 7.7×10^{-5} M during LTD_4-induced contraction, respectively. On the other hand, HSR-6071-induced relaxation of the isolated trachea was unaffected by the pretreatment with propranolol (3×10^{-7} M) (data not shown).

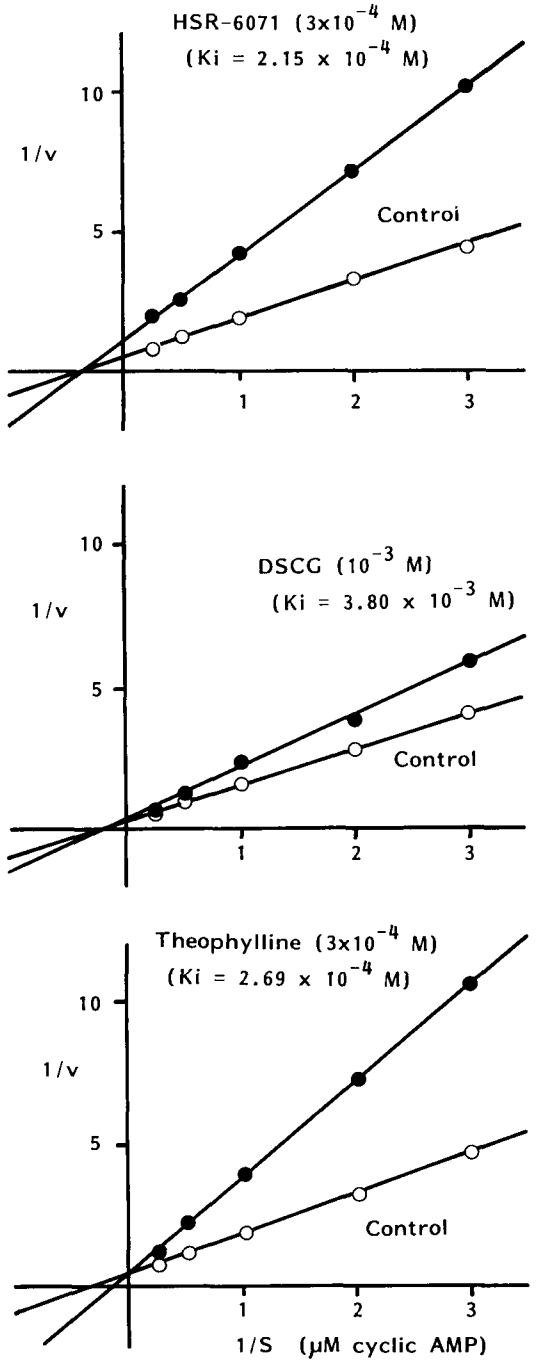

FIG. 4. Lineweaver-Burk plots for the effects of HSR-6071, DSCG and theophylline on cAMP-PDE from guinea-pig lung preparations. Each point represents the mean of 3 to 4 experiments.

Discussion

Bronchial asthma is considered as a typical atopic disease mediated by IgE antibody (Orange 1973). DSCG has been proved to be useful in the prophylactic treatment of allergic bronchial asthma (Howell & Altounyan 1967). In the experimental models, it has been found that DSCG possesses an inhibitory activity on the passive cutaneous anaphylaxis (PCA) and asthmatic respiratory response mediated by IgE-like antibody in rats (Goose & Blair 1969; Goto et al 1980). Therefore, it seems worthwhile to examine whether a putative anti-asthmatic agent inhibits PCA and experimental asthma. In the present experiments and in a previous report (Koda et al 1978), we confirmed that DSCG was effective in these experimental models in rats. However, DSCG had no effect on the asthmatic response mediated by IgE or IgG antibody in guinea-pigs. As for HSR-6071, this agent inhibited PCA (Makino et al 1989) and asthmatic response in rats, and IgE- and IgG-mediated bronchoconstriction in guinea-pigs. In this respect, the pharmacological properties of HSR-6071 were different from those of DSCG, but rather resembled those of amlexanox, which has recently been developed as a clinically useful agent (Kishimoto et al 1986).

Histamine and SRS-A (mixture of LTC_4, LTD_4 and LTE_4) are suggested to be major mediators for the induction of the asthmatic response mediated by IgE and IgG antibody in

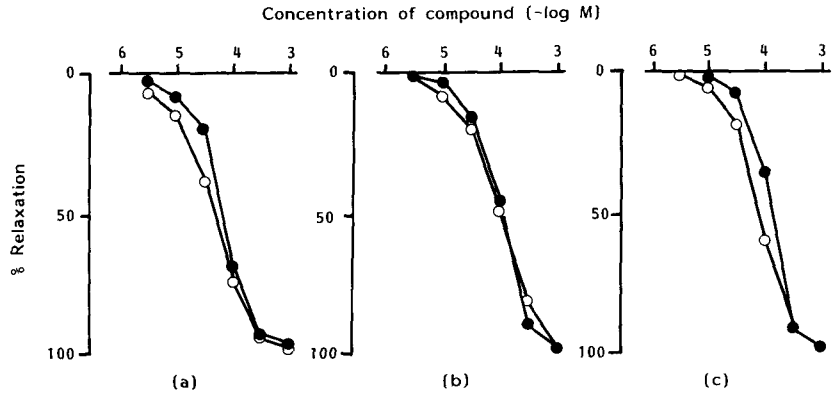

FIG. 5. Relaxing activity of HSR-6071 (-●-) and theophylline (-○-) in the quiescent state (a) and during contraction with 10^{-5} M histamine (b) and 10^{-8} M LTD_4 (c) of the guinea-pig isolated trachea. Each point represents the mean of 3 to 4 experiments.

guinea-pigs (Andersson 1982). Indeed, histamine and LTD_4 mimicked the antibody-mediated asthmatic response, which was inhibited by ketotifen, a histamine antagonist, and FPL-55712, an LTD_4 antagonist, respectively. In addition, histamine and LTD_4 were detectable after the allergic response in guinea-pig lung tissues. It seems likely, therefore, that these results support the suggestion described above. Since HSR-6071 as well as amlexanox inhibited the asthmatic responses mediated by IgE and IgG antibody in guinea-pigs, and release of LTD_4 and/or histamine from guinea-pig lung fragments, and exhibited the antagonistic action to LTD_4, the inhibitory action of these two agents may be at least partly due to the inhibition of mediator release in addition to their antagonistic action to SRS-A. However, it is necessary to investigate the effect of HSR-6071 on the generation of TXA_2 and TXA_2-induced airway responses, since TXA_2 is involved in LTD_4-induced bronchoconstriction in guinea-pigs (Omini et al 1981; Weichman et al 1982; Jones & Masson 1985).

It is well established that an increase in the intracellular cAMP level leads to the inhibition of allergic histamine release and the relaxation of smooth muscle. HSR-6071 was proved to be a noncompetitive inhibitor of cAMP-PDE, while theophylline was competitive. These two agents produced relaxation of the tracheal smooth muscle of the same magnitude. These pharmacological actions may contribute to produce anti-allergic action of HSR-6071.

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