

Effect of cinnarizine on IgE antibody-mediated experimental allergic reactions in guinea pigs

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The anti-allergic activity and mechanism of cinnarizine was investigated in guinea pigs. Nifedipine, a calcium antagonist, and tranilast, a potent, orally active anti-allergic agent, were used as comparative drugs. 1) Cinnarizine protected against fatal systemic anaphylactic shock in guinea pigs passively sensitized with IgE antibody. Cinnarizine reduced many of the features of severe respiratory disorders. Nifedipine and tranilast showed similar effects. 2) Cinnarizine and nifedipine inhibited the contractile response to antigen of sensitized tracheal smooth muscle when the challenge was carried out at low antigen concentrations. Tranilast showed a tendency to inhibit the antigen-induced contraction of tracheal smooth muscle. 3) Cinnarizine and nifedipine inhibited Ca-induced contraction in potassium-depolarized tracheal smooth muscle, tranilast had no effect. 4) Cinnarizine showed antagonistic action to the contraction by histamine or leukotriene D₄ (LTD₄) of tracheal muscle. Nifedipine showed similar antagonistic action, although its potency is lower than cinnarizine. Tranilast showed slight antagonistic action to LTD₄. 5) Antigen-induced release of histamine and slow reacting substance of anaphylaxis (SRS-A) from sensitized lung tissues was inhibited by nifedipine and tranilast but not by cinnarizine. 6) The release of histamine and SRS-A from lung tissues by calcium ionophore A23187 was inhibited by nifedipine and tranilast but not by cinnarizine. These results suggest that the anti-allergic action of cinnarizine is mainly due to the antagonistic action to allergic mediators and not by interfering with the release of mediators. Cinnarizine's mechanism seems to be related to its antagonistic action to Ca in smooth muscle, but not to the transport of Ca in releasing the anaphylactic chemical mediators in mast cells and other target cells.

Key words: anti-allergic agent; calcium antagonist; cinnarizine

Accepted for publication 4 November 1986

Cinnarizine is one of the classical histamine H₁ receptor antagonists. The drug is reported to be an inhibitor of transmembrane calcium (Ca) influx in smooth muscle (3, 4). These pharmacological properties should be beneficial for the treatment of allergic reaction since histamine and the Ca ion play important roles in many allergic conditions, especially asthma. In fact, there are some reports indicating the efficacy of cinnarizine in the treatments of allergic rhinitis and chronic asthma (1, 11, 15). Contrary to clinical investigational results, reports on the basic *in vitro* and animal pharmacology about anti-allergic activity of

cinnarizine are rarely found. In addition, the anti-allergic mechanism of cinnarizine has not been fully elucidated. Therefore, the study reported here was conducted to investigate the effect of cinnarizine on IgE antibody-mediated allergic reactions and its mechanism in guinea pigs.

MATERIAL AND METHODS

Drugs

Cinnarizine was kindly supplied by Eisai Pharmaceutical Co. Ltd., Tokyo, Japan. Nifedipine

was purchased from Bayer Pharmaceuticals, A. G., Postach, Federal Republic of Germany, and tranilast was supplied by Kissei Pharmaceutical Co. Ltd., Matsumoto, Japan. Cinnarizine and nifedipine were dissolved in dimethyl sulfoxide (DMSO). The concentration of DMSO against tissue was under 0.02%. Leukotriene (LT) D₄ was kindly supplied by Dr. S. Terao (Takeda Pharmaceutical Co. Ltd., Osaka, Japan).

Hartley guinea pigs weighing 300 to 350 g were used. The animals were purchased from Shizuoka Laboratory Animal center.

Sensitization

Guinea pigs were sensitized with antigen, benzyl-penicilloyl bovine- γ -globulin (BPO-BGG), and adjuvant, aluminium hydroxyde (Al(OH)₃), according to the method of Levine et al. (5, 6). Briefly, benzylpenicillin-K was dissolved in physiological saline and then gradually conjugated to bovine- γ -globulin under alkaline condition (pH 11.4–11.5). Excess benzylpenicillin was removed by dialysis against Tris-buffered saline (pH 8.2). Sensitization was performed by intraperitoneal injection of 1 ml of Tris-buffered saline containing 2 μ g of BPO-BGG and 1 mg of Al(OH)₃ monthly for 8 to 12 months. Sensitized animals were obtained more than 10 days after the last sensitization.

Passive systemic anaphylaxis (PSA)

PSA was induced by injection of antigen into the saphenous vein of guinea pigs which had been passively sensitized with anti-BPO-BGG IgE antibody 24 h before challenge. The amount of IgE antibody and antigen required to kill a guinea pig within 10 min was determined from results of preliminary experiments. The symptoms seen in shock were as follows: sneezing, coughing, ruffled fur, retching, convulsion, weakness, diminished tone, rales, prostration, and death. Since the severity of shock correlated well with the number of symptoms seen in the experiment with antigen, the evaluation of the general symptoms was expressed as four classes according to the number of above

symptoms, 0: asymptomatic, I: 1–3, II: 4–7, III: ≥ 8 , or death.

Contractile studies

Guinea pig tracheal smooth muscle preparations were used. The guinea pigs were stunned and exsanguinated. The trachea was excised, trimmed from excess tissue and cut longitudinally in the cartilage tissue part. The open trachea was cut in 10 segments. Five segments were tied together to form a chain and placed in an organ bath containing Tyrode's solution. Changes in tone of the preparation with 0.5 g initial resting tension were recorded isotonically (MEC, ME-4013, World Medical Co. Ltd.). In most experiments the drugs were incubated with the tissue for 5 min before the addition of antigen, CaCl₂, histamine or LTD₄. The contractile responses were expressed as a percentage of 10⁻⁷ g/ml carbacol-induced contraction. Cumulative concentration-contraction curves were generated for each tracheal chain by successively increasing the concentration of antigen, CaCl₂, histamine or LTD₄ in the bath in accordance with the method of Van Rossum (12). Each successive concentration increase was made at 5-min intervals. The contractile responses were again expressed as a percentage of the maximum control antigen, CaCl₂-histamine- or LTD₄-induced contraction.

Histamine and SRS-A release from guinea pig lung tissue

Lungs from sensitized or non-sensitized guinea pigs were chopped on a McIlwain tissue chopper and suspended in 10 times volume of Tyrode's solution. Histamine and SRS-A release were induced by incubation with antigen (10⁻⁵ g/ml) or calcium (Ca) ionophore A23187 (5 $\times$ 10⁻⁶ g/ml) at 37°C for 20 min. The amount of histamine in the incubation medium was assayed by a fluorescence method according to May et al. (7). The partial purified SRS-A fraction was assayed on stripped longitudinal smooth muscle of guinea pig ileum, which has been pretreated with atropine (5 $\times$ 10⁻⁷ M) and pyrilamine (10⁻⁸ M). By definition,

one unit (U) of SRS-A produces a contraction equivalent to 5×10^{-9} g/ml histamine. The partial purification of SRS-A was done by 80 % ethanol extraction and stepwise elution by different concentrations of ethanol from a SEP-PAK column (Waters Associates, Mass., USA). The SRS-A fraction was eluted by 70 % ethanol after washing the column with water and 35 % ethanol (pH 6.8) solution. In preliminary experiments, histamine, acetylcholine and serotonin were removed and more than 90 % LTC₄ and D₄ was recovered.

Statistics

Statistical analysis was performed using Student's t test.

RESULTS

Effect on PSA. The effect of the test agents on PSA is summarized in Table 1. Cinnarizine prevented fatal shock and reduced the appearance of general symptoms, especially respiratory system disorders. Nifedipine and tranilast showed similar effects.

Effect on antigen-induced contraction of tracheal muscle. The antigen-induced contraction of sensitized tracheal muscle was increased stepwise by the successive increase of antigen concentration in the bath. Maximum contraction by antigen was equivalent in amplitude to approximately 80–140 % of the maximum contraction caused by 10⁻⁷ g/ml carbacol. Pretreatment of tracheal smooth muscle with cin-

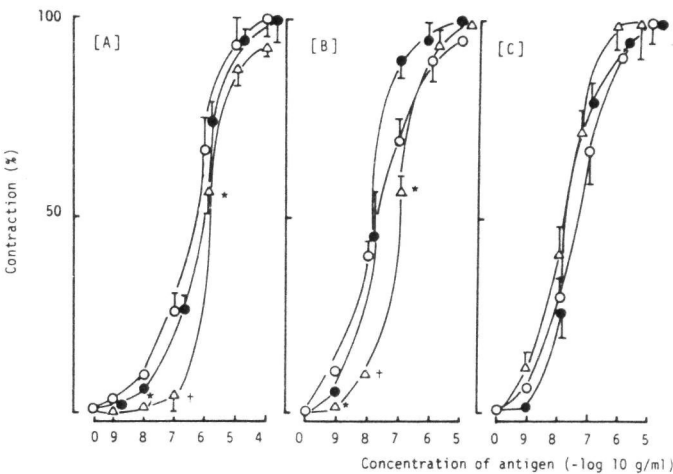


Fig. 1. Effect of cinnarizine [A], nifedipine [B] and tranilast [C] on antigen-induced contraction of sensitized guinea pig tracheal smooth muscle. Each point represents the mean of 8 to 12 experiments. Vertical bars mean standard error of experiments.
[A] ○: control, ●: 10⁻⁶ g/ml, △: 10⁻⁵ g/ml
[B] ○: control, ●: 10⁻⁶ g/ml, △: 10⁻⁵ g/ml
[C] ○: control, ●: 10⁻⁵ g/ml, △: 10⁻⁴ g/ml
P < 0.05, +: *P* < 0.01

narizine (10⁻⁵ g/ml) and nifedipine (10⁻⁵ g/ml) inhibited the amplitude of contractions caused by antigen at concentrations between 10⁻⁹ and 10⁻⁷ g/ml (Fig. 1). When the sensitized tracheal preparation was exposed to antigen at concentrations between 10⁻⁶ and 10⁻⁴ g/ml, however, the amplitude of the contraction was not affected. Tranilast at concentrations of 10⁻⁵ and 10⁻⁴ g/ml did not affect the antigen-induced contraction of tracheal muscle.

Effect on histamine- and LTD₄-induced contraction in tracheal muscle. On the non-sensitized guinea pig trachea, histamine elicited a concentration-

Table 1
Effect of cinnarizine, nifedipine and tranilast on passive systemic anaphylaxis in guinea pigs

Item	Control	Cinn (10 mg/kg)	Cinn (20 mg/kg)	Nife (20 mg/kg)	Tranilast (200 mg/kg)
% of lethality ^{1,2}	80 (12/15)	30 (3/10)	30 (3/10)	40 (2/5)	20 (1/5)
General symptom ³	III × 15	III × 5 II × 5	III × 6 II × 4	III × 3 II × 2	III × 2 II × 3

¹; Lethality was measured 60 min after antigen challenge.
²; Number in parentheses: dead animals/examined animals.
³; General symptom expresses score of symptom × number of animals expressed symptoms.

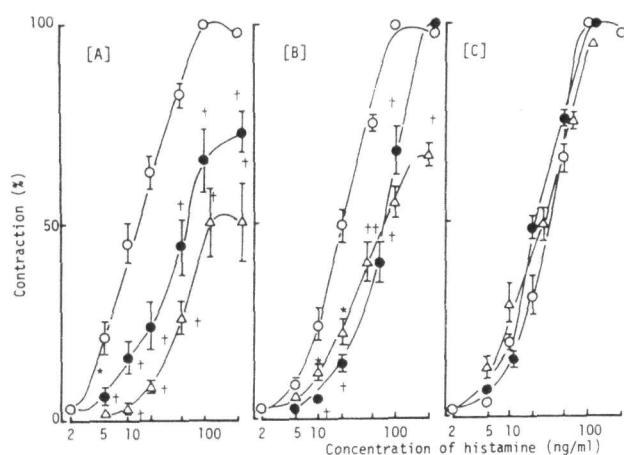


Fig. 2. Effect of cinnarizine [A], nifedipine [B] and tranilast [C] on histamine-induced contraction of guinea pig tracheal muscle. Each point represents the mean of 6 to 16 experiments. Vertical bars mean standard error of experiments.

[A] ○: control, ●: 10^{-6} g/ml, △: 10^{-5} g/ml
 [B] ○: control, ●: 10^{-6} g/ml, △: 10^{-5} g/ml
 [C] ○: control, ●: 10^{-5} g/ml, △: 10^{-4} g/ml
 $P < 0.05$, +: $P < 0.01$

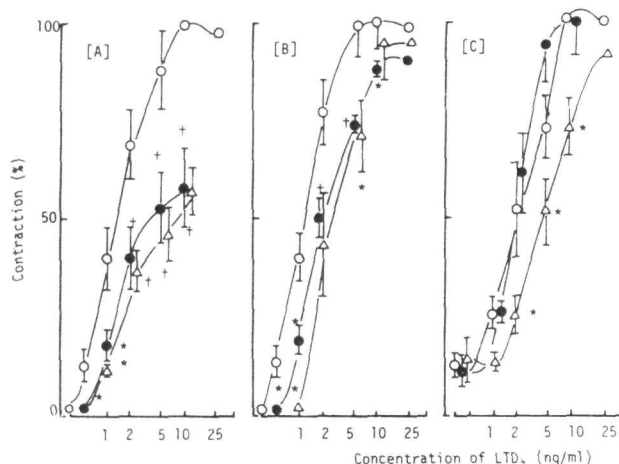


Fig. 3. Effect of cinnarizine [A], nifedipine [B] and tranilast [C] on LTD_4 -induced contraction of guinea pig tracheal smooth muscle. Each point represents the mean of 6 to 16 experiments. Vertical bars mean standard error of experiments.

[A] ○: control, ●: 10^{-6} g/ml, △: 10^{-5} g/ml
 [B] ○: control, ●: 10^{-6} g/ml, △: 10^{-5} g/ml
 [C] ○: control, ●: 10^{-5} g/ml, △: 10^{-4} g/ml
 $P < 0.05$, +: $P < 0.01$

dependent contraction over the concentration range 2×10^{-9} to 5×10^{-7} g/ml. The histamine-induced dose response curves were shifted to the right by pretreatment with cinnarizine (10^{-6} and 10^{-5} g/ml) and nifedipine (10^{-6} and

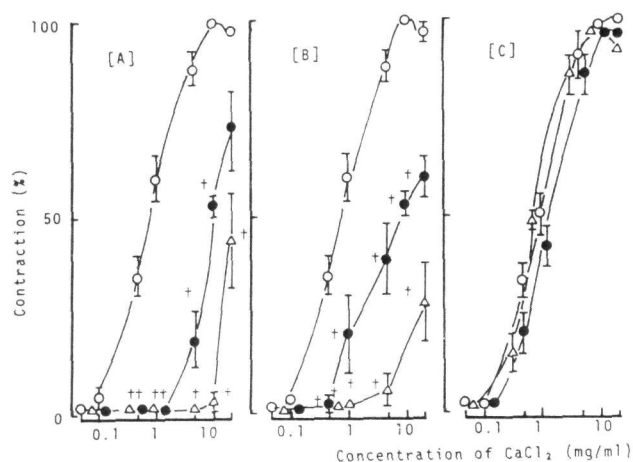


Fig. 4. Effect of cinnarizine [A], nifedipine [B] and tranilast [C] on CaCl_2 -induced contraction of K-depolarized guinea pig tracheal smooth muscle. Each point represents the mean of 6 to 16 experiments. Vertical bars mean standard error of experiments.

[A] ○: control, ●: 10^{-6} g/ml, △: 10^{-5} g/ml
 [B] ○: control, ●: 10^{-8} g/ml, △: 10^{-7} g/ml
 [C] ○: control, ●: 10^{-5} g/ml, △: 10^{-4} g/ml
 $P < 0.05$, +: $P < 0.01$

10^{-5} g/ml) (Fig. 2). Tranilast had no effect on histamine-induced contraction. LTD_4 -induced dose response curves were obtained between 5×10^{-10} and 2.5×10^{-8} g/ml. Cinnarizine (10^{-6} and 10^{-5} g/ml) significantly blocked the LTD_4 -induced contraction in a dose-dependent manner (Fig. 3). Nifedipine (10^{-6} and 10^{-5} g/ml) and tranilast (10^{-4} g/ml) also inhibited the LTD_4 -induced contraction.

Effect on Ca-induced contraction in K-depolarized tracheal muscle. It is generally accepted that Ca-induced contraction of depolarized muscle is regarded as an experimental model for Ca influx from the extracellular medium. In a series of experiments, effects of three drugs were studied on the Ca-induced contraction in guinea pig tracheal muscle. Tracheal muscle was incubated for 60 min in Ca-free Tyrode's solution and then for 6 min in a high K Tyrode's solution omitting Ca ions (high K Tyrode's solution was made by replacement of NaCl with KCl). Thereafter, a contraction was induced by cumulatively adding CaCl_2 (10^{-3} to 2×10^{-4} g/ml) to the medium in a concentration-dependent manner. While tranilast (10^{-5} and 10^{-4} g/ml) had no effect on Ca-induc-

ed contraction, cinnarizine (10^{-6} and 10^{-5} g/ml) and nifedipine (10^{-8} and 10^{-7} g/ml) significantly inhibited contractions.

Effect on antigen-induced histamine and SRS-A release. The amount of histamine released from sensitized guinea pig lung tissue was not affected by pretreatment of cinnarizine at concentrations between 10^{-7} and 10^{-5} g/ml (Table 2). Nifedipine (10^{-5} and 10^{-4} g/ml) and tranilast (10^{-4} g/ml) inhibited the release of histamine. SRS-A release was not affected by cinnarizine but was inhibited by both nifedipine (10^{-4} g/ml) and tranilast (10^{-4} g/ml).

Effect on Ca-ionophore A23187 induced histamine and SRS-A release. Ca-ionophore A23187 induced the release of approximately two-fold amounts of histamine and SRS-A when compared to those by antigen. Whereas nifedipine and tranilast

clearly inhibited the release of histamine and SRS-A, cinnarizine had no effect on the release of either mediator from lung tissue caused by A 23187 (Table 3).

DISCUSSION

The results reported here show the efficacy of cinnarizine on IgE antibody-mediated allergic reactions and its possible mechanism in guinea pigs.

The anti-allergic action of cinnarizine has been reported in some clinical studies in allergic rhinitis and chronic asthma (1, 11, 15). These two disorders may include an IgE antibody-mediated process for their onset and development. However, there is limited basic data or reference concerning the efficacy and mechanism of cinnarizine on IgE antibody-mediated allergic reactions. In the present study, we show the efficacy of cinnarizine on

Table 2
Effect of cinnarizine, nifedipine and tranilast on antigen-induced histamine and SRS-A release from sensitized guinea pig lung tissues

Treatment	Concentration (g/ml)	<i>n</i>	Histamine (ng/ml)	<i>n</i>	SRS-A (U/ml)
Control		5	23.5 ± 2.01	6	43.2 ± 2.08
Cinnarizine	10 ⁻⁷	5	28.8 ± 3.14	5	55.0 ± 5.65
	10 ⁻⁶	5	27.3 ± 4.60	5	51.2 ± 5.30
	10 ⁻⁵	5	25.7 ± 4.01		ND
Nifedipine	10 ⁻⁶	5	21.1 ± 0.99	6	41.5 ± 2.93
	10 ⁻⁵	5	14.0 ± 0.38*	6	42.1 ± 1.10
	10 ⁻⁴	5	5.5 ± 1.17**	6	18.9 ± 1.76**
Tranilast	10 ⁻⁵	5	21.6 ± 1.59	5	49.1 ± 2.45
	10 ⁻⁴	5	10.2 ± 0.30**	5	34.6 ± 1.39**

Each drug was incubated with lung tissues at 37°C for 30 min. ND: not done, * *P* < 0.05, ** *P* < 0.01.

Table 3
Effect of cinnarizine, nifedipine and tranilast on calcium ionophore A 23187-induced histamine and SRS-A release from guinea pig lung tissues

Treatment	Concentration (g/ml)	<i>n</i>	Histamine (ng/ml)	SRS-A (U/ml)
Control		10	46.5 ± 8.13	82.6 ± 11.20
Cinnarizine	10 ⁻⁶	9	45.7 ± 4.72	72.6 ± 6.32
	10 ⁻⁵	9	49.1 ± 6.32	83.0 ± 10.40
Nifedipine	10 ⁻⁶	10	36.1 ± 3.63	40.2 ± 3.78**
	10 ⁻⁵	10	28.4 ± 6.03*	39.3 ± 5.40**
Tranilast	10 ⁻⁵	10	31.7 ± 2.40*	42.4 ± 4.14**
	10 ⁻⁴	10	26.3 ± 4.54*	19.5 ± 3.30**

Each drug was incubated with lung tissues at 37°C for 30 min. * *P* < 0.05, ** *P* < 0.01.

IgE antibody-mediated systemic anaphylactic fatal shock and contraction of tracheal smooth muscle in guinea pigs. In addition, cinnarizine showed relief of many respiratory disorders during anaphylactic shock. These pharmacological actions suggest the utility of this drug as a remedy for atopic asthma. However, to date, cinnarizine has not been clinically investigated for the treatment of atopic asthma. Clinical investigation would be highly desirable.

As for the anti-allergic mechanism of cinnarizine, we present several hypotheses in the present report. The results suggest that the anti-allergic action of cinnarizine is mainly due to the antagonistic action towards anaphylactic chemical mediators and not due to its interfering with the release of mediators. In addition, this inhibitory mechanism seems to be closely related to calcium antagonist activity in the tracheal smooth muscle and mast cells. Since cinnarizine clearly inhibited Ca-induced contraction of tracheal smooth muscle but not the release of chemical mediators, the calcium antagonist action of cinnarizine is limited to the tracheal smooth muscle and not to the mast cells. This action is apparently different from the action of nifedipine. Nifedipine showed calcium antagonistic action in both smooth muscle and mast cells. These data suggest, therefore, that nifedipine is more beneficial than cinnarizine as a remedy for asthma. Since allergic asthma includes both Ca-dependent stimulation-excitation coupling and stimulation-secretion coupling processes, anti-asthmatic drugs with calcium antagonist activity need the inhibitory action to both processes (2, 10). We are now investigating new cinnarizine derivatives which possess antagonistic action to both calcium-dependent processes.

The anti-asthmatic activity of Ca antagonists has been discussed by many investigators (8, 9, 13, 14). However, the effectiveness of the agents on atopic, IgE antibody-mediated asthma is still inconsistent. When Ca antagonists which show high affinity to tracheal muscle have been identified, their real effectiveness as a remedy for asthma will be evaluated. Similarly, new cinnarizine derivatives with high affinity for bronchial muscle should be developed as a remedy for bronchial asthma.

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