

Effects of Flutropium on Experimental Models of Drug- and Allergy-Induced Rhinitis in Guinea Pigs

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Received August 13, 1990 Accepted December 21, 1990

ABSTRACT—The effects of flutropium on histamine (Hist)-induced increase in intranasal pressure in non-sensitized guinea pigs and nasal mucosa capillary permeability in passively sensitized guinea pigs were investigated. Flutropium (0.3%), atropine (0.3%), diphenhydramine (0.01%) and cimetidine (0.1%) were directly inhaled into the nasal cavities by an ultrasonic nebulizer for 20 min, followed by inhalation of Hist (0.1%) for 10 min. Flutropium, atropine and diphenhydramine had an inhibitory action on the Hist-induced increase in intranasal pressure in guinea pigs. Cimetidine had no effect on this system. In passively sensitized guinea pigs (the challenge was performed 48 hr after sensitization), a 0.1–1.0 mg/kg injection of flutropium (i.v.) dose-dependently inhibited the allergic nasal mucosa capillary permeability. Atropine (10 mg/kg, i.v.) had no inhibitory action on this system. These results suggest that inhalation into the nasal cavities and i.v. injection of flutropium are effective in experimental models of drug- and allergy-induced rhinitis of the guinea pig.

Typical symptoms of nasal allergy in humans are well-known to be mainly sneezing, hypersecretion and swelling of the nasal mucus membrane (1). The etiology of the development of allergic rhinitis is considered to be dependent on a mediator release from the sensitized mast cells in the subepithelial layer of the nasal mucosa (2). Histamine is one of the mediators of the nasal allergic reaction (3). It has been suggested that the nasal swelling induced by histamine is due to dilation of capacity vessels, congestion and local circulatory disorder in intranasal mucosa (2). The perfusion of antigen into the nasal cavities of sensitized rats and guinea pigs induced the IgE-mediated increase in nasal capillary permeability (4, 5). The therapy of allergic rhinitis has widely used antihistaminergic, antiallergic, anticholinergic and steroid drugs (6–9).

Recently, it has been reported by Gold et al. (10), Yu et al. (11) and Yamatake et al.

(12) that allergic bronchoconstriction is inhibited by atropine or vagal blockade. These results suggest that the vagal reflex plays an important role in various airway responses. It is known that the parasympathetic innervation of nasal mucosa plays an important role in the rhinitis (13). Flutropium bromide (flutropium) is a new antiasthma drug possessing the quarternary ammonium salt structure of an atropine derivative. In addition to the anticholinergic action, flutropium has antihistaminergic and antiallergic actions (14–16). However, there are very few experimental rhinitis models in guinea pigs. In the present study, we attempted to devise an experimental rhinitis model in guinea pigs and to investigate the effects of flutropium on experimental models of histamine-induced increase in intranasal pressure and allergic rhinitis in guinea pigs.

MATERIALS AND METHODS

Animals

Male Hartley guinea pigs were purchased from Japan SLC, Inc. (Hamamatsu, Japan). Food and water were given ad libitum.

Effects on histamine (Hist)-induced increase in intranasal pressure

Guinea pigs weighing 425–950 g were used. The intranasal pressure was continuously measured by the modified method described by Noguchi et al. (5). Guinea pigs were anesthetized with sodium pentobarbital (30 mg/kg, i.p. and 10 mg/kg, s.c.). The animals were fixed in a supine position and spontaneously respired through a tracheal cannula inserted into the trachea. The esophagus was ligated with a thread to interrupt the air flow across the cavity. A polyethylene cannula (Natsume, SP-110) was inserted into the nasopharynx from the side of the larynx, and the other side of the polyethylene cannula was connected to a Y-shaped cannula. One side of the Y-shaped cannula was connected to an artificial respirator (Shinano, SN-480-7) and the other side was connected to a pressure transducer (San-ei, LPU-0.1). Air was flowed into the nasal cavities through the nasopharynx with the artificial respirator (500 ml/min). The intranasal pressure was continuously measured with a pressure transducer. The oral cavities were filled

with glycerin-soaked absorbent cotton and was closed with Alon-alpha (Konishi) to protect a pressure leakage. The systemic blood pressure was measured with a pressure transducer (Toyo-Boldwin, SPU-300), and heart rate was measured with a cardiometer (San-ei, N4778) using the systolic blood pressure as the trigger. Histamine was inhaled for 10 min into the nasal cavities by an ultrasonic nebulizer (Nihon Kohden, TUR-3200). The ultrasonic nebulizer was placed between the nasopharynx and artificial respirator (Fig. 1). Flutropium, atropine, diphenhydramine and cimetidine were inhaled for 20 min prior to histamine inhalation. The consumption rate of inhaled drug solution was about 0.3 ml/min. The experimental schedule is shown in Fig. 2. The observation was carried out for 30 min from the onset of histamine inhalation into the nasal cavities of the guinea pig.

Effects on allergic rhinitis

Guinea pigs weighing 450–630 g were used. Guinea pig antiserum was prepared according to the method described by Misawa et al. (16). Responses of the experimental allergic rhinitis were measured according to the method described by Kojima et al. (4). Guinea pigs were passively sensitized (0.1 ml/100 g body weight, i.p.) with ovalbumin antiserum (1/30-fold dilutions with saline). The challenge was performed 48 hr after sensitiza-

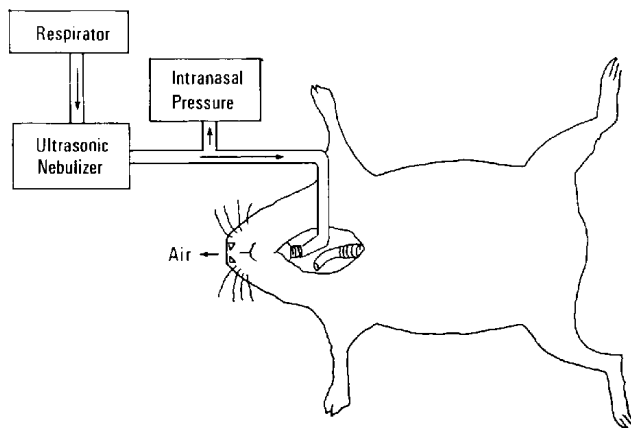
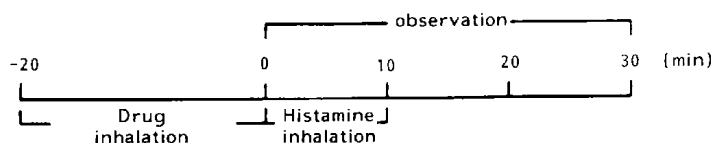


Fig. 1. Diagram of the preparation for measuring the intranasal pressure.

1) Intranasal pressure



2) Nasal mucosa capillary permeability

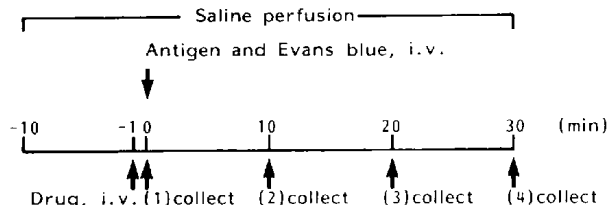


Fig. 2. Experimental schedules.

tion. Guinea pigs were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The animals were spontaneously respired through a tracheal cannula inserted into the trachea. The esophagus was ligated with a thread to interrupt perfusate flow across the cavities. A polyethylene cannula was inserted into the nasopharynx from the side of the larynx and the other side of the polyethylene cannula was connected to a perfusion pump (Tokyo-Rikakikai, MP-3). Perfusate (37°C, saline) was perfused at the rate of 0.25 ml/min. Antigen (1 mg/ml, 0.1 ml/100 g body weight) was injected i.v. into the cervical vein, followed by Evans blue (8%, 0.5 ml/100 g body weight), i.v. injection. Drugs were given i.v. 1 min prior to antigen challenge. The leakage fluid from the nasopharynx was collected 4 times at intervals of 10 min (Fig. 2) and was centrifuged at 3000 rpm for 15 min. The amount of dye of the supernatant was estimated at 620 nm with a photometer (Hitachi, 200-10). The total amount of dye for 30 min after antigen injection was calculated.

Drugs

Flutropium bromide (Boehringer Ingelheim, W-Germany), diphenhydramine hydrochloride (Tokyo Kasei, Japan), atropine sulfate (Wako

Pure Chemicals, Japan), cimetidine (Sigma, U.S.A.), pentobarbital-Na (Tokyo Kasei, Japan), ovalbumin (Sigma, U.S.A.), histamine dihydrochloride (Wako Pure Chemicals, Japan), azelastine hydrochloride (extract from Azeptin; Eisai, Japan) and Evans blue (Tokyo Kasei, Japan) were used. All drugs were dissolved in saline solution.

Statistical analysis

All values were expressed as the mean with S.E. Statistical evaluation was performed by one-way analysis of variance after the Bartlett test, followed by the Dunnett or Scheffé test for multiple comparison.

RESULTS

Effects on histamine (Hist)-induced increase in intranasal pressure

The response slowly recovered to the beginning level after the end of inhalation of Hist (0.1%) (Fig. 3). Inhalation of Hist (0.03, 0.1 and 0.3%) into the nasal cavities caused a concentration-related increase in intranasal pressure (Fig. 4). On the other hand, an inhalation of saline for 10 min into the nasal cavities had no effect on intranasal pressure and the other measured parameters. More-

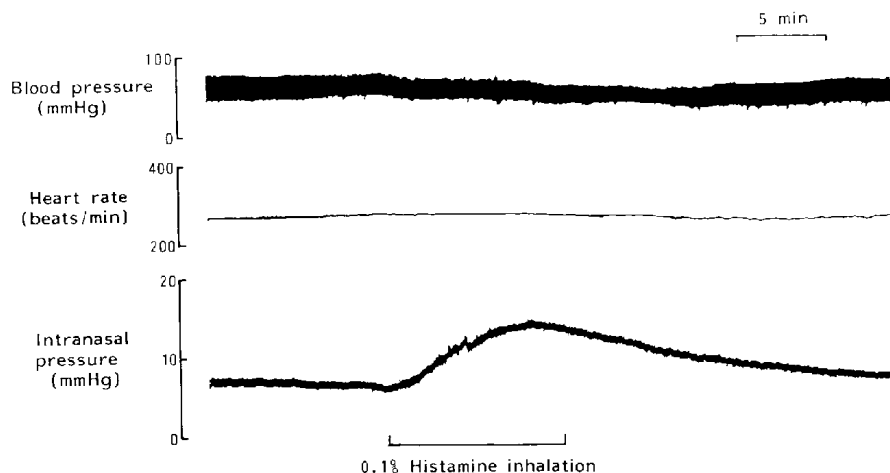


Fig. 3. Typical response to inhalation of 0.1% histamine into the nasal cavities of the guinea pig.

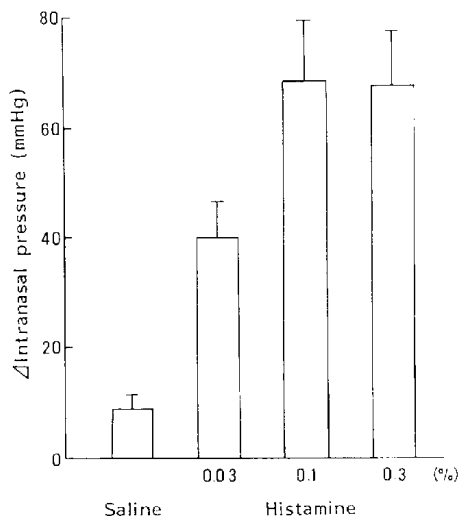


Fig. 4. The change in intranasal pressure by a 10-min inhalation of histamine (0.03, 0.1 and 0.3%). The ordinate shows the total integrated intranasal pressure for 30 min period from the onset of histamine inhalation into the nasal cavities of the guinea pig. Each column represents the mean with S.E. of 4 animals.

over, inhalation of a 0.03 or 0.1% solution of Hist into the nasal cavities had no effect on systemic blood pressure and heart rate. However, inhalation of a 0.3% solution of Hist produced hypotension in a few preparations. From the above results, a 0.1% histamine solution was used in this study. Inhalations of flutropium (0.03, 0.1 and 0.3%) and

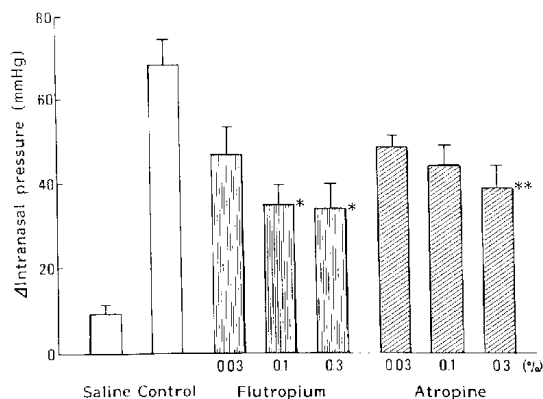


Fig. 5. Effects of flutropium and atropine on the histamine-induced increase in intranasal pressure in guinea pigs. The drugs were inhaled for 20 min before a 10-min inhalation of histamine (0.1%). Each column represents the mean with S.E. of 5 to 9 animals. *, **: Significantly different from the control at $P < 0.05$ and $P < 0.01$, respectively.

atropine (0.03, 0.1 and 0.3%) into the nasal cavities decreased the Hist-induced increase in intranasal pressure (Fig. 5). The inhibitory action of flutropium was slightly stronger than that of atropine. Inhalation of flutropium and atropine had no effect on systemic blood pressure and heart rate. Figure 6 shows the effects of diphenhydramine (H_1 -antagonist) and cimetidine (H_2 -antagonist) inhalations on Hist-induced increase in intranasal pressure.

A 0.003–0.03% solution of diphenhydramine concentration-dependently inhibited the Hist-induced increase in intranasal pressure, but a 0.03 and 0.1% solution of cimetidine had no effect. Inhalation of diphenhydramine (0.03%)

had no effect on systemic blood pressure and heart rate, but, inhalation of cimetidine (0.1%) caused hypotension in half of the treated animals.

Effects on allergic rhinitis

A 0.1–1 mg/kg, i.v.-injection of flutropium dose-dependently inhibited the dye leakage due to antigen (Fig. 7). Atropine had no effect at a dose of 10 mg/kg, and a 30 mg/kg showed a weak inhibitory action (Fig. 7). Diphenhydramine (0.1 mg/kg) and azelastine (3 and 10 mg/kg) showed an inhibitory action (Fig. 7). Figure 8 shows the total amount of dye for 30 min period after antigen injection. Flutropium at 0.3 mg/kg caused inhibition equivalent to that produced by 0.1 mg/kg of diphenhydramine. On the other hand, 3 and 10 mg/kg azelastine caused inhibition equivalent to those produced by 0.3 and 1 mg/kg of flutropium, respectively.

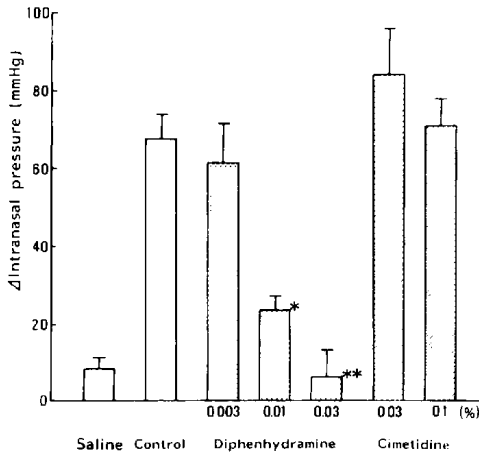


Fig. 6. Effects of diphenhydramine and cimetidine on the histamine-induced increase in intranasal pressure in guinea pigs. Each column represents the mean with S.E. of 4 to 9 animals. Other explanations are as in Figs. 4 and 5.

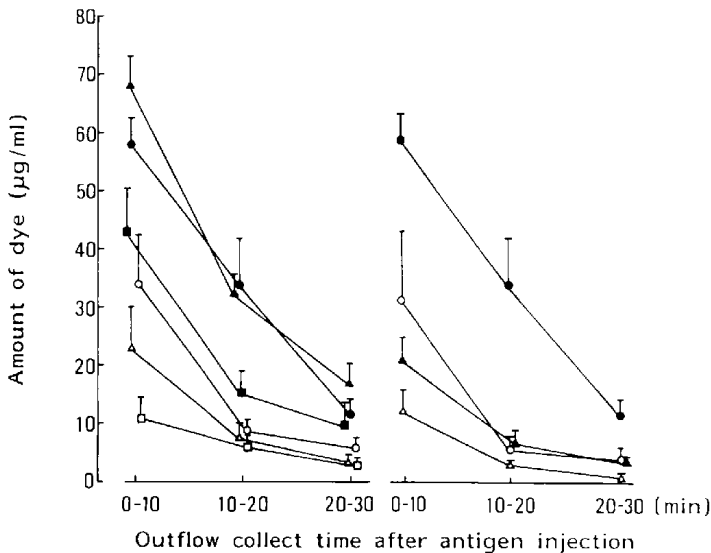


Fig. 7. Effects of flutropium (left: ○, 0.1 mg/kg; △, 0.3 mg/kg; □, 1.0 mg/kg), atropine (left: ▲, 10.0 mg/kg; ■, 30.0 mg/kg), azelastine (right: ○, 3.0 mg/kg; △, 10.0 mg/kg) and diphenhydramine (right: ▲, 0.1 mg/kg) on the increase in nasal mucosal capillary permeability in passively sensitized guinea pigs. The drugs were given i.v. 1 min before antigen challenge. Each point represents the mean with S.E. of 5 to 6 animals. ●, control.

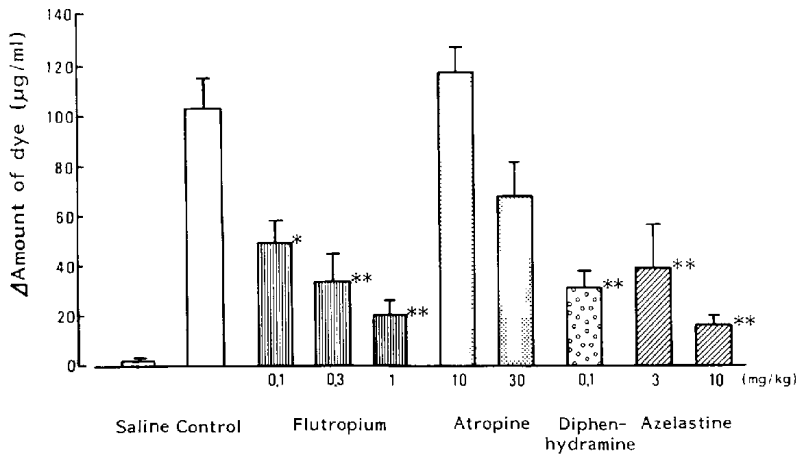


Fig. 8. Effects of flutropium, atropine, diphenhydramine and azelastine on the increase in nasal mucosa capillary permeability in passively sensitized guinea pigs. The ordinate shows the total integrated amount of dye for a 30 min period after antigen injection. Each column represents the mean with S.E. of 5 to 6 animals. *, **: Significantly different from the control at $P < 0.05$ and $P < 0.01$, respectively.

DISCUSSION

Flutropium has been used in the therapy of bronchial asthma (17, 18) and rhinitis (19–21). In addition to an anticholinergic action (14), flutropium can stabilize the cell membrane (16) and has an antihistaminergic action (14, 15). The anticholinergic action of flutropium was stronger and longer lasting than that of atropine (14). Flutropium has been reported to cause bronchodilation in experimental models of drug- and allergy-induced bronchoconstrictions in dogs (14). However, no pharmacological study on the effects of flutropium on rhinitis has hitherto been performed. In the present study, we investigated the effect of flutropium on experimental rhinitis models of the guinea pig.

However, there are very few experimental rhinitis models in guinea pigs. Loux (22) and Malm (23) measured the change of intranasal pressure as an index for the rhinitis of dogs or cats. The experimental rhinitis models using guinea pigs have been reported by Noguchi et al. (5) and Ukai et al. (24), in which a drug solution was infused into the nasal cavities from the trachea or dropped into the intranasal cavities, respectively. Recently, a new experimental rhinitis model in rats using a modi-

fication of a Konzett-Rössler apparatus has reported by Misawa (25), in which the drug solution was inhaled into the nasal cavities. It is well-known that guinea pigs are generally hypersensitive to histamine as compared to rats. Moreover, guinea pigs have been widely used in allergic experiments. In the present study, we devised a model for continuously measuring the increase in intranasal pressure induced by histamine inhalation in guinea pigs. Clinically, flutropium is given topically as an aerosol into the nasal cavities in the therapy of rhinitis and we investigated the effect of flutropium inhalation. Inhalation of flutropium (0.1 and 0.3%) and atropine (0.3%) significantly inhibited the increase in intranasal pressure by histamine inhalation. The increase in intranasal pressure induced by histamine is considered to be due to swelling of nasal mucosa (dilation of capacity vessels or congestion) mediated by histamine receptors in the blood vessels of nasal mucosa (1, 2). It is well-known that the blood vessels contain histamine H_1 - and H_2 -receptors. Diphenhydramine (histamine H_1 antagonist) inhibited the increase in intranasal pressure by histamine inhalation, but cimetidine (histamine H_2 antagonist) did not. These findings suggest that histamine H_1 receptors may participate in in-

tranasal pressure elevation induced by histamine. It is considered that the mechanism of action of flutropium and atropine are due to direct H_1 -receptor antagonistic actions in the blood vessels of the nasal mucosa (26). The inhibitory action of flutropium was slightly stronger than that of atropine. The different inhibitory action between flutropium and atropine may be due to the difference of the antihistaminergic activities as has already been reported by Yanaura et al. (14).

In the allergic rhinitis study, we used a model for measuring the dye leakage into the nasal cavities by i.v. injection of antigen in passively sensitized guinea pigs. It has been reported by Kimura et al. (27) that orally administered flutropium was poorly absorbed in the gastrointestinal tract. Misawa et al. (16) reported that i.v. injection of flutropium showed antiallergic action against the 48 hr homologous PCA in guinea pigs. In the present study, flutropium was given i.v. Azelastine, an antiallergic drug, has been given orally in clinical use. However, in the present study, this drug was administered i.v. for comparison with flutropium. Flutropium (0.1 and 0.3 mg/kg) significantly inhibited the dye leakage due to antigen. In contrast, atropine at a dose of 10 mg/kg had no inhibitory action, but 30 mg/kg showed a weak inhibitory action. On the other hand, diphenhydramine (0.1 mg/kg) and azelastine (3 and 10 mg/kg) significantly inhibited the allergic rhinitis. Recently, it has been reported by Yanaura et al. (14), Mizuno and Ohno (15) and Misawa et al. (16) that flutropium has antihistaminergic and antiallergic actions. Moreover, it has been reported by Okuda (6) and Boursquet et al. (7) that anti-allergic and antihistaminergic (H_1 -antagonists) drugs are effective in patients with allergic rhinitis.

From the above results, we conclude that flutropium with antihistaminergic and anti-allergic actions may be an effective therapeutic drug in patients with allergic rhinitis. Moreover, it is considered that the experimental models of histamine-induced rhinitis in guinea pigs can be applied for the development of

therapeutic drugs for rhinitis.

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