Effects of Muscarinic Antagonists on Experimental Nasal Secretion in Guinea Pigs

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ABSTRACT—The effects of muscarinic antagonists on acetylcholine (ACh)- and histamine-induced nasal secretion were investigated in guinea pigs. Inhalations of flutropium (0.01 to 0.3%) and atropine (0.03 to 0.3%) into the nasal cavities dose-dependently inhibited the nasal secretion induced by ACh. The inhibitory action of flutropium was slightly stronger than that of atropine. Inhalations of pirenzepine (0.3%) and gallamine (0.3%) had no effect on the ACh-induced nasal secretion. However, 4-DAMP dose-dependently inhibited the nasal secretion induced by ACh. Inhalations of flutropium (0.3%) and diphenhydramine (0.3%) showed a similar inhibitory action on the histamine-induced nasal secretion. These results suggest that 1) inhalation into the nasal cavities of flutropium was effective in experimental model of ACh- and histamine-induced nasal secretion, 2) M₃ cholinergic receptors may be dominant in the nasal secretion induced by ACh and 3) the experimental model of drug-induced nasal secretion in guinea pigs used in the present study can be employed to develop therapeutic drugs for nasal secretion.

The chemical mediators released as a result of allergic reactions play an important role at the onset of the symptoms of nasal allergy: sneezing, hypersecretion and swelling of the nasal mucosa (1). Histamine is considered to be one of the most important chemical mediators in the occurrence of these allergy symptoms (2). Sneezing may be due to stimulation of the sensory nerve with histamine (3). Swelling of the nasal mucosa may be due to stimulation of the nasal vasculature with chemical mediators, congestion and local circulatory disorder of the nasal mucosa (1), while hypersecretion may be due to a leakage from the nasal vasculature by histamine (4) or a product from the nasal glands by the neural reflex release of acetylcholine (ACh) from the parasympathetic nerve terminals (5). Also, it is well-known that the parasympathetic nervous system plays an important role in the regulation of secretion from the nasal glands. The presence of muscarinic receptors have been described in the nasal glands of rats (6) and humans (7). It has been reported that the nasal secretion by electrical stimulation of the parasympathetic nerve in animals and the methacholine-induced nasal secretion in humans with nasal allergy can be inhibited by anticholinergic drugs (7–10).

Flutropium bromide (flutropium) is a new anti-asthma drug possessing the quarternary ammonium salt structure of an atropine derivative (11, 12). In addition to anticholinergic action, flutropium has anti-
histaminergic and anti-allergic actions (13, 14). It is reported that flutropium given topically into the naso-oral tract as an aerosol shows a clinical efficacy in patients with nasal allergy and rhinorrhea (15–18). However, no pharmacological study of the effect of flutropium on nasal secretion have hitherto been performed. A good model for a nasal secretion study has not yet been reported in laboratory animals. In the present study, therefore, we attempted to devise a model of experimental nasal secretion in guinea pigs and to investigate the effects of flutropium using this model. Furthermore, we observed the effects of other anti-cholinergic drugs.

MATERIALS AND METHODS

Animals

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

Preparations of drug-induced nasal secretion

Guinea pigs weighing 370–580 g were used. Guinea pigs were anesthetized with urethane (1.6 g/kg, s.c.). The animals were fixed in a supine position and spontaneously respired through a cannula inserted into the trachea. The esophagus was ligated with a thread to interrupt 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 an artificial respirator (Shinano, SN-480-7). The room air was flowed into the nasal cavities through the nasopharynx with the artificial respirator at a volume of 10 ml/breath and a frequency of 30 breaths/min. All studies were performed in an experimental room under a temperature of 22 ± 2°C and humidity of 55 ± 15%. The oral cavities were filled with glycerin-soaked absorbent cotton to prevent any contamination by saliva and were closed with Alon-alpha (Konishi) to protect against pressure leakage. The systemic blood pressure was measured with a pressure transducer (Toyo-Boldwin, SPU-300), and heart rate was measured with a cardiotachometer (San-ei, N4778) using the systolic blood pressure as the trigger.

Experimental schedules

ACh or histamine were inhaled for 5 min into the nasal cavities by an ultrasonic nebulizer (Nihon Kohden, TUR-3200). The ultrasonic nebulizer was placed between the nasopharynx and artificial respirator. The consumption rate of inhaled drug solution was 0.3 ± 0.004 ml/min (n = 153). Flutropium, atropine, pirenzepine, gallamine, 4-DAMP and diphenhydramine were inhaled for 10 min prior to acetylcholine (or histamine) inhalation. The observation was carried out for 25 min from the onset of acetylcholine (or histamine) inhalation. The animal was used only for one experimental schedule. The exocrine secretion from the nasal mucosa was collected by a piece of aluminium foil (80 × 100 mm: Nihon Seihaku) and absorbed by a rectangular piece of paper (50 × 60 mm: Kimwipe, S-200), which was preweighed. The piece of aluminium foil was placed on the outside of the nasal cavities. In addition, after 25 min, any exocrine secretion that adhered around the nose was wiped off by a piece of paper and the paper was again weighed.

Drugs

Flutropium bromide (Boehringer Ingelheim, Germany), diphenhydramine hydrochloride (Tokyo Kasei, Japan), atropine sulfate (Wako Pure Chemicals, Japan), gallamine triethiodide (Sigma, U.S.A.), 4-diphenylacetoxy-N-methylpiperidine metiodide (4-DAMP; Research Biochemicals, U.S.A.), acetylcholine chloride (Ovisort; Daichi Seiyaku, Japan), sodium pentobarbital (Tokyo Kasei, Japan), histamine dihydrochloride (Wako Pure Chemicals, Japan), urethane (Aldrich Chemical Company, U.S.A.) and pirenzepine hydrochloride (extract from Gastrozepin; Tanabe Seiyaku, 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

Inhalation of ACh (1, 2 and 3%) into the nasal cavities caused a concentration-related increase in nasal secretion (Table 1). An inhalation of 3% ACh solution produced a continuous hypotension, and it took more than 60 min for a recovery to the initial level. From these results, we decided to use a 2% ACh solution in this study.

Inhalations of 1, 1.5 and 2% histamine into the nasal cavities caused a concentration-related increase in nasal secretion (Table 1). Inhalations of 1, 1.5 and 2% histamine produced a continuous hypotension of about 30% in all animals, and it took more than 60 min for a return to the initial level. A 2% histamine solution was used in this study.

On the other hand, an inhalation of saline for 10 min into the nasal cavities had no effect on systemic blood pressure and heart rate (data not shown).

Table 1. Change in nasal secretion by acetylcholine and histamine inhalation into the nasal cavities of the guinea pig

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (%)</th>
<th>Nasal secretion (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td>12 ± 6</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>1</td>
<td>30 ± 10</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>76 ± 6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>70 ± 9</td>
</tr>
<tr>
<td>Histamine</td>
<td>1</td>
<td>48 ± 15</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>68 ± 17</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>102 ± 7</td>
</tr>
</tbody>
</table>

Acetylcholine (1, 2 and 3%) and histamine (1, 1.5 and 2%) were inhaled for 5 min. The nasal secretion was collected for 25 min from the start of acetylcholine (or histamine) inhalation. Each value represents the mean ± S.E. of 4 to 12 animals.

Inhalations of flutropium (0.01 to 0.3%) and atropine (0.03 to 0.3%) into the nasal cavities concentration-dependently inhibited the ACh-induced increase in nasal secretion (Fig. 1). Flutropium (0.3%) and atropine (0.3%) had an inhibitory action of about 90% and 75%, respectively. The inhibitory action of flutropium was slightly stronger than that of atropine. Inhalations of pirenzepine (0.3%) (M1-antagonist) and gallamine (0.3%) (M2-antagonist) had no effect, but inhalation of 4-DAMP (0.01 to 0.3%) (M3-antagonist) concentration-dependently inhibited the ACh-induced increase in nasal secretion (Fig. 2). 4-DAMP (0.3%) showed an inhibitory action of about 65%. Furthermore, inhalation of diphenhydramine (0.3%) had no effect on this system (Fig. 3). On the other hand, pentobarbital at the dose of 3 mg/kg (i.v.) (pretreatment) showed an inhibitory action of about 63% (Fig. 3). Inhalations of flutropium, atropine, pirenzepine, gallamine, 4-DAMP and diphenhydramine at the concentration used in this study had no effect on systemic blood pressure.

![Fig. 1. Effects of flutropium and atropine on the acetylcholine-induced increase in nasal secretion in guinea pigs. Drugs were inhaled for 10 min before a 5 min inhalation of acetylcholine (2%). Each column represents the mean with S.E. of 5 to 12 animals. *: Significantly different from the control at P < 0.05 and P < 0.01, respectively.](image-url)
Fig. 2. Effects of pirenzepine (Pir.), gallamine (Gal.) and 4-DAMP on the acetylcholine-induced increase in nasal secretion in guinea pigs. Each column represents the mean with S.E. of 5 to 9 animals. **: Significantly different from the control at P < 0.01. Other explanations are as in Fig. 1 and Table 1.

Fig. 3. Effects of diphenhydramine (Diph.) and pentobarbital (Pent.) on the acetylcholine-induced increase in nasal secretion in guinea pigs. Diphenhydramine was inhaled for 10 min and pentobarbital was given by i.v. injection at 1 min before a 5-min inhalation of acetylcholine (2%), respectively. Each column represents the mean with S.E. of 5 animals. **: Significantly different from the control at P < 0.01. Other explanations are as in Table 1.

Fig. 4. Effects of diphenhydramine (Diph.), flutropium (Flu.) and pentobarbital (Pent.) on the histamine-induced increase in nasal secretion in guinea pigs. Diphenhydramine and flutropium were inhaled for 10 min before a 5-min inhalation of histamine (2%). Pentobarbital was given by i.v. injection at 1 min before a 5-min inhalation of histamine (2%). Each column represents the mean with S.E. of 5 animals. **: Significantly different from the control at P < 0.01. Other explanations are as in Table 1.

DISCUSSION

The etiology of the development of nasal allergy is thought to be dependent on a mediator release from mast cells after the reaction between antigens and the mast cells in the nasal mucosa (1). The hypersecretion is well-known to be one of the mainly typical symptoms of nasal allergy in humans (1). The hypersecretion in nasal allergy is thought to be due to the following: 1) the activation of the nasal glands by ACh released from the parasympathetic nerve (vidian nerve) terminals as the results of the parasympathetic nerve reflex with histamine, other chemical mediators and
irritant stimulation to the sensory nerve on the nasal mucosa, 2) the direct effects of ACh on muscarinic receptors in the nasal glands and 3) the leakage of plasma from the nasal vasculature by the direct effects of histamine and other chemical mediators on the nasal vasculature (4). It has been generally thought that the nasal secretion is mainly secreted from the nasal glands (3).

Also, it has been generally accepted that the autonomic nervous system plays an important role in nasal allergy which is thought to be caused by an enhancement of the parasympathetic nerve activity in the nasal mucosa. It has been reported that the nasal secretion by methacholine and electrical stimulation of the vidian nerve in animals can be inhibited by vidian neurorectomy and anti-cholinergic drugs, respectively. Furthermore, it has been reported that the neurorectomy of the vidian nerve and the treatment of anti-cholinergics blocked the hypersecretion in patients with nasal allergy (4, 7).

In the present study, we divided experimental nasal secretion models induced by drug inhalations into the nasal cavities in guinea pig and measured directly the amount (weight) of nasal secretion as the index of evaluation. The coefficient of correlation between 2% ACh and 2% histamine inhaled animals' body weights and nasal secretions were 0.566 and 0.269, respectively, which were not significant.

On ACh-induced nasal secretion, the inhibitory action of flutropium was slightly stronger than that of atropine. It is considered that the difference of inhibitory action between flutropium and atropine may be dependent upon the anti-cholinergic activities as has been reported by Yanaura et al. (13). Inhalation of pirenzepine (M1-antagonist) and gallamine (M2-antagonist) had no effect on the hypersecretion induced by ACh, while inhalation of 4-DAMP (M3-antagonist) dose-dependently inhibited the nasal secretion. Bloom et al. (19) reported that 50% inhibition of the vagal induced increase in pulmonary resistance of pirenzepine was about 8-fold greater than the equieffective dose of atropine. Inhalation of 3.0% pirenzepine showed a weak inhibitory action of about 29% (data not shown); however, it was not a statistically significant difference from the control. On the other hand, inhalation of 3.0% gallamine resulted in the death of the guinea pigs. This indicates that inhalation of 0.3% gallamine may be thought of as a high concentration. These results suggest that M3-cholinergic muscarinic receptors are dominant for the nasal secretion induced by ACh in guinea pigs.

Pentobarbital (3 mg/kg, i.v.) showed about 60% inhibition of the nasal secretion induced by ACh. On histamine-induced nasal secretion, pentobarbital showed about 85% inhibition on the nasal secretion. Pentobarbital more effectively inhibited the nasal secretion induced by histamine than that of ACh. Tsuchiya et al. (20) has been reported that pentobarbital (3 mg/kg, i.v.) reduced the vagal reflex tracheal constriction. The higher centers may affect the reflex airway constriction. In the present study, a low dose of pentobarbital (3 mg/kg, i.v.) reduced the nasal secretion induced by inhalation of ACh and histamine into the nasal cavities. Hukuhara (21) has reported that a low dose of pentobarbital inhibited neuronal discharges of the respiratory center without cardiovascular effects. These results may indicate the involvement of a nasal reflex in the nasal secretion of guinea pigs. Pentobarbital may affect the reflex complement of the nasal secretion.

Inhalations of diphenhydramine had no effect on the ACh-induced nasal secretion, but had an inhibitory effect on the histamine-induced nasal secretion. Shelhamer et al. (22) reported that the nasal secretion induced by histamine may be mainly due to the leakage of plasma from the nasal vasculature. However, in the present study, the nasal secretion induced by histamine may be due to the leakage of plasma from the nasal vasculature and the product from the nasal glands via the nasal reflex. The inhibitory effect of diphenhydramine may be due to the antagonization of H1 receptors on the nasal vasculature and the nasal
mucosa. On the other hand, the nasal secretion induced by ACh involves the direct effects on muscarinic receptors and the indirect effects via the nasal reflex in the nasal glands.

The inhibitory effect of flutropium on the histamine-induced nasal secretion may be due to the antagonistic action towards H1 receptors on the nasal vasculature and those on the nasal mucosa and to muscarinic receptors on the nasal glands. On the other hand, the inhibitory effect of flutropium on the ACh-induced nasal secretion may be due to the antagonistic action against muscarinic receptors on the nasal mucosa and the nasal glands. Ishibe et al. (23) has been reported that the number of muscarinic receptors of the human nasal mucosa increases in patients with nasal allergy. These results suggest that the anticholinergic drugs may be more effective for nasal hypersecretion.

These results suggest that 1) flutropium may be an effective drug in hyperesthetic rhinitis; 2) M3-cholinergic receptors may be dominant for the nasal secretion in guinea pigs; and 3) the experimental model of drug-induced nasal secretion in guinea pigs used in the present study can be employed to develop therapeutic drugs for nasal secretion.

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