Effect of Oxitropium Bromide (Ba253) on Increased Airway Resistance Induced by Various Agonists and Antigen in the Guinea Pig

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Abstract—Effects of oxitropium bromide (Ba253), which was administered by inhalation, on the resting and stimulus-induced airway resistance were examined in the artificially ventilated guinea pig and compared with those of ipratropium bromide (Sch1000), atropine and isoproterenol. Results obtained were as follows: 1) Ba253 as well as other reference compounds hardly affected the resting resistance. 2) Ba253 strongly and persistently inhibited the acetylcholine (ACh)-induced resistance. Sch1000 caused a similar but relatively weaker inhibition than Ba253. Either atropine or isoproterenol caused only a transient inhibition. 3) The increase in resistance induced by histamine, serotonin, leukotriene D4 or antigen was prevented by Ba253. Atropine, Sch1000 and isoproterenol also inhibited these reactions, but the effects and the duration were generally weaker and shorter than those of Ba253. 4) Repeated inhalations of Ba253 for 7 days did not influence the inhibition of the ACh-induced increase in airway resistance by this drug. However, isoproterenol tended to attenuate the suppression of the resistance by the drug. From these results, it is suggested that Ba253 is a useful inhalant drug for asthma.

It has long been recognized that the symptoms in asthmatic patients improve when they inhale substances derived from belladonna plants by smoking. Elliott and Reid (1) reported that increased expiratory volume as a consequence of the dilated airway smooth muscle and decreased heart rate and systemic actions as generally seen with the anticholinergics were observed in healthy subjects when they inhaled the smoke of a herb containing belladonna alkaloids. Ipratropium bromide (Sch1000), a derivative with an isopropyl at the N position of atropine, has been reported to have strong anticholinergic action. Since Sch1000 is characterized as showing low absorption from the digestive tract because it has increased polarity relative to the parent compound due to changing the tertiary amine to a quaternary ammonium, the drug has been expected to be useful for asthma therapy with less general systemic action than the usual anticholinergics (2–6). Recently, oxitropium bromide (Ba253), a derivative with an ethyl group at the N position of scopolamine, has been developed as a new drug that has a more potent therapeutic effect on asthma than the present anticholinergics (7). This compound has a quaternary ammonium in the molecule as well.

We previously reported the effects of Ba253 on isolated pulmonary smooth muscle and the release of anaphylactic chemical mediators from lung fragments (8). In the present study, we examined the effects of inhaled Ba253 on the increases in airway resistance induced by various stimuli in guinea pigs and compared them with those of atropine, Sch1000 and isoproterenol.
Materials and Methods

Drugs

(8r) - 6β,7,β - Epoxy - 8 - ethyl - 3α[(-)] - tropoyoxy - 1αH,5αH - tropanium bromide (oxitropium bromide, Ba253) and ipratropium bromide (Sch1000, Nippon Boehringer Ingelheim, Kawanishi), atropine sulfate (Merck, Darmstadt) and /-isoproterenol D-bitartrate (Nakarai Chem., Kyoto) were used. Those drugs were dissolved in distilled water and used as their bases.

Animals

Three weeks-old male Hartley guinea pigs (Shizuoka Lab. Animal Ctr., Hamamatsu) were purchased. The animals were used at 3 to 6 weeks of age after breeding under the conditions of solid feed (LABO RG-RO, Nihon Nosan, Tokyo), temperature of 22±1.5°C and humidity of 55±15%.

Antigen and antiserum

Benzylpenicilloyl bovine gamma globulin (BPO-BGG) as the antigen and the guinea pig antiserum against it, as described elsewhere (8), were used.

Passive sensitization

The guinea pigs were passively sensitized with i.v. injection of 0.25 ml/animal of the antiserum and used in the experiments 2 days later.

The airway resistance change induced by various stimuli

1. Preparation of the artificially ventilated animal: For cannulation of the airway, tracheotomy was performed on the guinea pig under anesthesia with sodium pentobarbital (Abbott Lab., North Chicago; 20 mg/kg, i.p.). Artificial ventilation (Respirator: Model 680, Harvard App., Southnatick; 4 ml/stroke, 40 strokes/min) was started at the time of interrupted spontaneous respiration by additional i.p. injection of 40 mg/kg of sodium pentobarbital. The airway resistance in the forced inspiration was recorded on the recticorder (RJG-4124, Nihon Kohden, Tokyo) via the bronchospasm transducer (No. 7020, Ugobasile, Camerio) and amplifier AB-621G, Nihon Kohden, Tokyo). The airway resistances were evaluated in terms of percent of bronchoconstriction by each stimulus before drug treatment or by rating as maximal (100%) a response obtained with the total occlusion of the tracheal cannula.

2. Inhalation system of the drug in the artificially ventilated animal: A schematic diagram of the inhalation apparatus is shown in Fig. 1. In this apparatus, the drug solution is pumped with a peristaltic pump (MP-3, EYELA, Tokyo Rikakikai, Tokyo. 10 ml/min) to the nebulizer equipped inside the cylindrical glass container (20 cm in height and 15 cm in diameter), and mist was produced by a compressed air jet from the air compressor (SC-72, Hitachi Koki, Tokyo, 2 kg/cm²). The mist was given to the artificially ventilated animal by inhalation via the respirator. The content of the mist in the air sprayed by the nebulizer was 0.53 ml/11 l air/min).

3. Inhalation conditions for drug solution: Evans blue solution of 5% or various concentrations instead of the drug solution was given to the guinea pig through the inhalation apparatus for 30 min or various periods. At the appropriate time, the guinea pig was exsanguinated and the ventilation was halted. Following perfusion with physiologic saline through the pulmonary artery, the lung was isolated. Evans blue inhaled into the lung was extracted and determined according to the method of Katayama et al. (9), with minor modification, using 1.5 ml/g wet tissue of 1.2 N KOH for lysis of the tissue. The required concentration of the drug in the solution and the duration of inhalation were determined on the basis of the amount of Evans blue inhaled.

4. Effect on the resting airway resistance: Effects of inhalation of 10 μg/animal of Ba253, Sch1000 and atropine or 1 μg/animal of isoproterenol on the resting airway resistance were investigated over a period of 90 min.

5. Effect on agonist- and antigen-induced increases in airway resistance: Effects of inhalation of the drugs on the acetylcholine (ACh)-, histamine-, serotonin- or leukotriene (LT)D₄- and antigen-induced increase in airway resistance were investigated in nonsensitized and sensitized guinea pigs, respectively. Inhaled doses were 10 μg/animal for Ba253, Sch1000 or atropine and 1 μg/animal for isoproterenol.

6. Effect of drug inhalation for 7 consecu-
**Fig. 1.** Schematic diagram of inhalation apparatus in the artificially ventilated guinea pig.

**Fig. 2.** Schematic diagram of inhalation apparatus in the conscious guinea pig.
tive days: Effects of 7-day repeated inhalation of Ba253, Sch1000, atropine or isoproterenol on the inhibition by the respective drugs of the ACh-induced increase in airway resistance under artificial ventilation were investigated. For repeated administration, the same nebulizer as shown in Fig. 1 was used, and the conscious guinea pig was allowed to inhale the drug mist introduced into the 28-l plastic chamber. The animal was fixed at the neck on a supporting board, and the nose of the animal was thrust into the chamber through the aperture (Fig. 2). The conditions for inhalation of an appropriate amount of the drug were determined by measuring the amount of Evans blue inhaled, as described under “3. Inhalation conditions for drug solution”.

The experiment on the ACh-induced increase in airway resistance as described in the context of “5. Effect on agonist- or antigen-induced increase in airway resistance” was performed 2 days after the repeated drug inhalation.

7. Statistical analysis: Statistical analysis was performed by Student’s t-test, and values ≤0.05 were considered significant.

Results

Effect on the various agonist- or antigen-induced increases in airway resistance

1. Determination of the amount of inhaled drug in the artificially ventilated animal: Figure 3 shows the time course-inhaled amount (left panel) and the inhaled amount (right panel) of Evans blue in the respiratory organ of the artificially ventilated guinea pig when 5% Evans blue was inhaled for various times and 0.5–5% Evans blue inhaled 30 min, respectively. As seen from the figure, there was a positive correlation between the time of inhalation or Evans blue concentration for a fixed inhalation time and the inhaled amount, the correlation coefficient being as high as 0.97 or 0.95.

Based on the amount of Evans blue inhaled, a 0.5% solution of Ba253, Sch1000 or atropine (10 μg/animal) and a 0.05% solution of isoproterenol (1 μg/animal) were given in the form of a mist to the artificially ventilated animal for 8.5 min.

To determine the dose-dependence of the

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**Fig. 3.** Correlation of inhaled amounts of Evans blue in the pulmonary organ and inhalation time (5% Evans blue) or various concentrations of Evans blue inhalation (30 min inhalation) in the artificially ventilated guinea pig. Each point represents the mean of 10 animals.
Effect of Ba253 on Airway Resistance

The effect of Ba253 and Sch1000 on the ACh-induced increase in airway resistance, the test drug was used at concentrations from 0.005 to 0.5% (inhaled amounts to be 0.1-10 μg/animal), with an inhalation time of 8.5 min.

2. Effect on the resting airway resistance: Effects of Ba253, Sch1000, atropine and isoproterenol on the resting airway resistance are shown in Fig. 4. Inhalation of the test drugs did not exert any significant effect on the resistance.

3. Effect on the agonist-induced increase in airway resistance: ACh: Effects of inhalation pretreatment with the test drugs on the ACh (10 μg/kg/time, i.v.)-induced increase in airway resistance are shown in Fig. 5.

The ACh-induced increase in airway resistance was significantly inhibited for 4 hr after inhalation of Ba253 or Sch1000, the inhibition rate still being about 40 and 50%, respectively, at 4 hr after the inhalation. Significant inhibition was also observed after inhalation of atropine and isoproterenol, but the inhibition by these drugs, unlike those by Ba253 or Sch1000, became weaker with time; the airway resistance became almost comparable to that in the control animals at about 120 and 50 min after inhalation of atropine and isoproterenol, respectively; and thereafter, the ACh-induced increase in airway resistance tended to become gradually larger than the control.

Figure 6 illustrates the dose-dependent effects of Ba253 and Sch1000 on the ACh-induced increase in airway resistance. Neither Ba253 nor Sch1000 at the dose of 0.1 μg/animal suppressed the resistance. Significant suppression was observed at 5–120 min and 30–110 min after inhalation of 1 μg/animal of Ba253 and Sch1000, respectively, the suppression by Ba253 being stronger than that by Sch1000.

Histamine: Effects of inhalation pretreatment with the test drugs on the histamine (3 μg/kg/time, i.v.)-induced increase in airway resistance are shown in Fig. 7.

Significant suppression of the airway resistance was observed for 60 min after the inhalation of Ba253, from 10 to 60 and 120 min after the inhalation of Sch1000, and for 30 min after the inhalation of isoproterenol. Atropine suppressed the resistance for 60 min after inhalation, although the suppression was insignificant.

Serotonin: Effect of inhalation pretreatment with the test drugs on the serotonin (2 μg/kg/time, i.v.)-induced increase in airway resistance is shown in Fig. 8.

Figure 8 illustrates the dose-dependent effects of Ba253 and Sch1000 on the serotonin-induced increase in airway resistance. Neither Ba253 nor Sch1000 at the dose of 0.1 μg/animal suppressed the resistance. Significant suppression was observed at 5–120 min and 30–110 min after inhalation of 1 μg/animal of Ba253 and Sch1000, respectively, the suppression by Ba253 being stronger than that by Sch1000.
Fig. 5. Effect of aerosol administration of Ba253, Sch1000, atropine and isoproterenol on the acetylcholine (10 µg/kg/time, i.v.)-induced increase in airway resistance in the artificially ventilated guinea pig. Each point represents the mean of 10 animals. Significant differences were also observed after treatment with Ba253, Sch1000 and atropine (P<0.001 at 0–20 min), and isoproterenol (P<0.001 at 0–10 min, P<0.05 at 20 min).

Fig. 6. Effect of aerosol administration of Ba253 and Sch1000 on the acetylcholine (10 µg/kg/time, i.v.)-induced increase in airway resistance in the artificially ventilated guinea pig. Each point represents the mean of 10 animals. ●: Control, △: Ba253 (0.1 µg/animal); ▲: Ba253 (1 µg/animal); Δ: Ba253 (10 µg/animal); ■: Sch1000 (0.1 µg/animal); □: Sch1000 (1 µg/animal); ▼: Sch1000 (10 µg/animal).
Fig. 7. Effect of aerosol administration of Ba253, Sch1000, atropine and isoproterenol on the histamine (3 μg/kg/time, i.v.)-induced increase in airway resistance in the artificially ventilated guinea pig. Each point represents the mean of 10 animals.

Fig. 8. Effect of aerosol administration of Ba253, Sch1000, atropine and isoproterenol on the serotonin (2 μg/kg/time, i.v.)-induced increase in airway resistance in the artificially ventilated guinea pig. Each point represents the mean of 10 animals.
resistance are shown in Fig. 8. Atropine and isoproterenol significantly suppressed the resistance for 10 min after the inhalation. Ba253 and Sch1000 tended to suppress the resistance until 90 min after inhalation, but the suppression was not significant.

LTD$_4$: Effects of inhalation pretreatment of the test drugs on the LTD$_4$ (0.4 μg/kg, i.v.)-induced airway resistance are shown in Figs. 9 and 10. Inhalation of Ba253, atropine or isoproterenol at 10 min before LTD$_4$ injection produced relatively strong suppression of

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**Fig. 9.** Effect of aerosol administration of Ba253, Sch1000, atropine and isoproterenol on the leukotriene (LT) D$_4$ (0.4 μg/kg, i.v.)-induced increase in airway resistance in the artificially ventilated guinea pig. Test drugs were given 10 min before LTD$_4$ injection. Each point represents the mean of 10 animals.

**Fig. 10.** Effect of aerosol administration of Ba253, Sch1000, atropine and isoproterenol on the leukotriene (LT) D$_4$ (0.4 μg/kg, i.v.)-induced airway resistance in the artificially ventilated guinea pig. Test drugs were given 60 min before LTD$_4$ injection. Each point represents the mean of 10 animals.
the LTD₄-induced increase in airway resistance. On the other hand, Sch1000 exerted no effect (Fig. 9). Inhalations of Ba253, Sch1000 and atropine 60 min before LTD₄ injection, produced relatively strong suppression of the resistance; Isoproterenol also produced suppression, although the suppression was slightly weaker (Fig. 10).

Antigen: Effects of inhalation pretreatment with the test drugs on the antigen (20 µg/animal, i.v.)-induced increase in airway resistance in the passively sensitized guinea pig are shown in Fig. 11. Ba253, Sch1000, atropine or isoproterenol tended to suppress the resistance, but the suppression was insignificant.

Effect of repeated inhalations of the drugs

1. Determination of the inhaled amount of the drugs in the conscious animal: The amount of Evans blue inhaled was investigated in the spontaneously breathing guinea pigs that inhaled the mist of 2% Evans blue instead of the test drugs. Guinea pigs weighing 510–520 g were allowed to inhale the mist for 30 min. The amount of Evans blue inhaled was 244±2 µg/animal (N=7). In the preliminary experiments, the amount of Evans blue inhaled under spontaneous breathing in the atmosphere containing the mist of Evans blue solution of a fixed concentration for a fixed period was linearly related with the body weight. According to this, to attain the inhalation of Ba253, Sch1000 and atropine at the dose of 10 µg/animal/day and isoproterenol of 1 µg/animal/day, 0.2% solutions of the former three drugs and a 0.02% solution of isoproterenol were applied in the form of an aerosol for 13 min/500 g body weight/day for 7 consecutive days. Control animals to the respective, repeatedly inhaled drug animals were treated in the same way with an aerosol of distilled water.

2. Results: Two days after the drug inhalation treatment of 7 consecutive days, the artificially ventilated guinea pigs were forced to inhale the respective drugs and then i.v. administered with 10 µg/kg of ACh to measure the increase in airway resistance. Effects of pretreatment with these drugs on the ACh-induced increase in airway resistance are shown in Fig. 12. No significant difference was observed between the group given

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Fig. 12. Influence of repeated administration of Ba253, Sch1000, atropine and isoproterenol on the suppression by respective drugs of the acetylcholine (10 μg/kg/time, i.v.)-induced increase in airway resistance in the artificially ventilated guinea pig. Animals were pretreated with the inhalation of Ba253 (▲), Sch1000 (■), atropine (●), isoproterenol (◆) or distilled water (★, △, □, ○ and ◆) for 7 consecutive days. On the 9th day, they were submitted to the assay using the acetylcholine-induced increase in airway resistance. Control animals were treated with an aerosol of distilled water instead of the drug solution at the ACh-induced increase in the airway resistance. The increase in the airway resistance by ACh was expressed as a percentage of that by ACh before inhalation of drug solutions or distilled water.

Discussion

It is well-known that the respiratory organ of the guinea pig is a good model of the human one in terms of the attack organ in anaphylaxis and the reactivity of the airway smooth muscle to various chemical mediators. In this paper, the influence of Ba253 on the respiratory reaction by various stimuli in the guinea pig was investigated in vivo.

The resting airway resistance under the artificially ventilated condition was not influenced by inhalation of Ba253. It is interesting that, unlike the results obtained from the in vitro experiment using the isolated airway smooth muscle (8), the in vivo resting airway resistance was neither increased by Sch1000 nor decreased by isoproterenol inhalation. Sch1000 made approaches to the bronchiole mainly from the serous membrane side in the isolated lung parenchyma, whereas in the inhalation experiment, it acted through the mucosal membrane; this may have been the main reason for the discrepancy between the in vitro and in vivo results. In addition, it has been generally recognized that the airway resistance does not greatly depend on the bronchiole but predominantly reflects the resistance in the main airway (10). A reason why isoproterenol inhalation did not decrease the resting airway resistance in the artificial ventilation may be that β-adrenergic stimulants such as isoproterenol minimally affect the large airway under the condition of mechanically compulsive inspiration as used in Ba253, Sch1000 or atropine for 7 consecutive and the group given distilled water for 7 consecutive days. However, repeated inhalation of isoproterenol tended to attenuate the suppression of the resistance by the drug.
Effect of Ba253 on Airway Resistance

Inhalation of Ba253 inhibited the ACh-induced increase in airway resistance strongly and persistently, and this inhibition was stronger than that produced by Sch1000 and much stronger than that produced by atropine. Thus, there was no difference in the inhibitory action of these anticholinergic agents on the ACh-induced airway response between in vitro (contraction of the isolated airway smooth muscle) (8) and in vivo (airway resistance). It is interesting that, different from in vitro results, inhalation of Ba253 strongly inhibited the histamine- and LTD4-induced increases in airway resistance, and it tended to inhibit the serotonin-induced increase in airway resistance. From the in vitro results from the isolated airway smooth muscle experiment (8), it was not considered that direct inhibition of the smooth muscle contraction by Ba253 was greatly responsible for the relatively strong inhibitory action by the drug on the increases in airway resistance induced by these mediators, except for ACh. It has been reported that histamine (11), serotonin (12, 13) and LTD4 (14) contract the airway smooth muscle not only by direct receptor-stimulation but also by stimulating the irritant receptor which is present in the subepithelial intracellular space. The stimulation of the irritant receptor results in the release of ACh to contract the airway smooth muscle from the efferent vagus fiber ending. Therefore, the inhibition of the mediator-induced airway resistance by Ba253 is presumed to be due to competition for the receptor between Ba253 and the released ACh, and thus the in vivo results may have been greatly different from the in vitro results. The same mechanism may be involved in the inhibition of antigen-induced airway resistance by Ba253 because major anaphylactic chemical mediators in the guinea pig have been reported to be histamine and slow reacting substance of anaphylaxis (SRS-A, peptide LTs) (15). As expected, 10 μg/kg of Ba253 inhibited the antigen-induced increase in airway resistance to a degree comparable with 1 μg/kg of isoproterenol. However, it was not significantly different from the control group because the increased resistance was relatively variable from animal to animal.

Inhalation of Ba253 and Sch1000 inhibited all of the examined stimulus-induced increases in airway resistance more strongly and more persistently than atropine did. This may be explained by the fact that the former two drugs, which are both quaternary ammonium compounds, are less lipophilic and thus, less absorbable so that they may be retained locally for a longer period. Inhalation of isoproterenol markedly but transiently inhibited the stimulus-induced increases in airway resistance, strongly suggesting that isoproterenol may readily be absorbed through the mucosal membrane and metabolized.

Anticholinergic agents have been used clinically for prevention of asthmatic attacks and acknowledged to require a considerable time to exert their effect, usually 2 to 4 weeks. Also disodium cromoglycate, tranilast and the like that inhibit the release of anaphylactic chemical mediators to show antiallergic action are generally recognized to require a considerable time to exert their effect. The reason for this remains unknown, but repeated administration of a drug might induce tolerance of the subject to the drug. Therefore, we investigated possible development of tolerance to Ba253 because this drug will also be used by repeated administration. Ba253 as well as Sch1000 and atropine did not cause any remarkable decrease in the sensitivity to the drug after repeated administration for 7 consecutive days. Although the effect of repeated administration of Ba253 on the number of ACh receptors in the tissue was not studied, this effect is supposed to be small because muscarinic agonists but not anticholinergic drugs have been reported to decrease Bmax on the muscarinic ACh receptors (16).

It has been reported that there is no difference in the density of β-receptors in leukocytes between patients with asthma who are not treated with β-stimulants and normal subjects. However, a decreased density of β-receptors has been observed in asthmatic patients who are treated with β-stimulants (17). In the present study, a tendency of decreased sensitivity to isoproterenol was observed after 7-day inhalation of this drug.

Taken these results together, it is expected
that Ba253 may not only alleviate the hyper-reactive state of the airway observed commonly in allergic patients but also be effective in the treatment of endogenous bronchial asthmatic attacks caused by non-specific stimulation and in the treatment of allergic asthma because vagus excitement may be involved in the antigen-induced bronchoconstriction. The effect of Ba253 has been suggested to be stronger than that of Sch1000.

The antiasthmatic effect of Ba253 is expected to become therapeutically stronger when the drug is administered in combination with a β-stimulant, a xanthine derivative, an antiallergic drug, a steroid or the like of which the mechanism of action is obviously different from that of Ba253.

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