A New Bronchial Asthma Model Using Calcium Ionophore A23187 in Guinea Pigs

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Abstract—We attempted to develop a nonimmunologically induced asthma model using the calcium ionophore A23187. Inhalation of A23187 (0.001–0.005%) for 5 min in male Hartley guinea pigs caused a marked bronchoconstriction in a dose-dependent manner with negligible effect on systemic blood pressure. The A23187-induced bronchoconstriction was strongly inhibited by chlorpheniramine and FPL-55712. These results indicate that an asthma-like bronchoconstriction was induced by inhalation of A23187 in guinea pigs, and the main chemical mediators involved in this response would be histamine and peptidoleukotrienes.

IgE-dependent mediator secretion from mast cells is accompanied by the increase in intracellular concentration of free calcium (1). Calcium ionophore A23187 (A23187), which increases calcium transport across cell membrane, has been widely used in vitro experiments to stimulate the release of chemical mediators from mast cells (2).

Stimulation of isolated guinea pig lung preparations with A23187 releases histamine and other mediators of anaphylaxis (3–5). However, most studies using A23187 have been carried out in vitro, and thus little information has been available concerning in vivo responses to A23187.

In the present study, we applied A23187 to induce a bronchoconstriction in vivo in guinea pigs. Furthermore, we investigated the effects of chlorpheniramine, a histamine H1-antagonist, and FPL-55712, a peptidoleukotriene antagonist, on the bronchoconstriction.

Male Hartley guinea pigs weighing 350 to 450 g, purchased from Tokyo Laboratory Animals Science Co., were used. Animals were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and immobilized with decamethonium bromide (initial dose of 0.2 mg/kg, i.v. and supplemental dose of 0.1 mg/kg when necessary). The animals were placed in the supine position and ventilated artificially through a tracheal cannula at a frequency of 70 breaths/min. Respiratory volume was adjusted at the beginning of the experiment so that ventilation overflow was 0.5–0.7 ml in each animal.

In anesthetized guinea pigs, the bronchomotor tone was measured by a modification of the Konzett-Rossler method (6). The lung was inflated at a fixed volume of air at room temperature and humidity under a constant pressure (6 cmH2O). Ventilation overflow was continuously recorded with the combination of a pneumotachograph (MFP-1T, Nihon Kohden) and an integrator (RFJ-5, Nihon Kohden), and this served as an index of change in airway resistance. Systemic arterial blood pressure was monitored with a pressure transducer (MPU-0.5, Nihon Kohden) and an integrator (RFJ-5, Nihon Kohden), and this served as an index of change in airway resistance. Systemic arterial blood pressure was monitored with a pressure transducer (MPU-0.5, Nihon Kohden) and an integrator (RFJ-5, Nihon Kohden), and this served as an index of change in airway resistance. Systemic arterial blood pressure was monitored with a pressure transducer (MPU-0.5, Nihon Kohden) and an integrator (RFJ-5, Nihon Kohden), and this served as an index of change in airway resistance. Systemic arterial blood pressure was monitored with a pressure transducer (MPU-0.5, Nihon Kohden) and an integrator (RFJ-5, Nihon Kohden), and this served as an index of change in airway resistance.

A23187 was given for 5 min by aerosolizing the A23187 solution (total output volume from the nebulizer, about 0.4 ml) contained in a plastic cylindrical chamber which was introduced into an ultrasonic nebulizer (TUR-3000, Nihon Kohden) (7). The ultrasonic...
nebulizer with the plastic chamber was placed in the respiratory circuit for the aerosolized mist to be inhaled into the airways at each time of ventilation. Immediately after the end of 5-min inhalation of A23187, the intratracheal lumen was gently cleaned with a twisted paper to remove the inhaled solution and airway secretion inside the lumen.

The drugs used were calcium ionophore A23187 (Sigma), histamine dihydrochloride (Wako Pure Chemicals), leukotriene D$_4$ monomethylester (Funakoshi), FPL-55712 (Fisons) and chlorpheniramine maleate (Tokyo Kasei). A23187 was dissolved in dimethylsulfoxide/saline (50v:50v). FPL-55712 was dissolved in distilled water. The other drugs were dissolved in saline.

All values were expressed as the mean with S.E. Statistical significance of difference was determined by Student's t-test.

A23187 at concentrations of 0.001, 0.0025 and 0.005% was inhaled for 5 min. The time course of the change in ventilation overflow induced by inhalation of A23187 is shown in Fig. 1. The increase in ventilation overflow induced by A23187 at a concentration of 0.001% was weak and variable. The treatment with A23187 at concentrations of 0.0025 and 0.005% caused a marked increase in ventilation overflow with only negligible effects on systemic blood pressure and heart rate. Ventilation overflow began to increase in 1–2 min after the start of inhalation, and the increase reached the peak by 20–25 min after the start of inhalation, which lasted for at least 30 min following the end of inhalation. The maximal changes in ventilation overflow at concentrations of 0.001, 0.0025 and 0.005% were 0.29±0.12 ml (N=5), 1.10±0.12 ml (N=9) and 1.37±0.30 ml (N=9), respectively. Inhalation of the vehicle (dimethylsulfoxide/saline) had no substantial effect on ventilation overflow.

Chlorpheniramine at a dose of 1 mg/kg, i.v., was administered 5 min before the inhalation of A23187. FPL-55712 at a dose of 2 mg/kg/min, i.v., was infused through the jugular vein, for 15 min from 5 min before the inhalation of A23187 to 5 min after the end of inhalation. Chlorpheniramine and FPL-55712 inhibited the increase in ventilation overflow induced by inhalation of A23187 (0.0025%) (Fig. 2). Inhibition rates by drugs were calculated by the integrated increase in ventilation overflow (AUC) for 20 min after the start of the inhalation of A23187. The inhibition rates of chlorpheniramine and FPL-55712 were by 52.1% (N=6) and 64.7% (N=5), respectively. The inhibitory effects of
chlorpheniramine and FPL-55712 were both significant. The doses of chlorpheniramine and FPL-55712 almost abolished the increase in ventilation overflow induced by histamine at a dose of 2.5 μg/kg, i.v., and leukotriene D₄ at a dose of 5 μg/kg, i.v., respectively.

A bronchial asthma attack is known to be initiated not only by inhalation of some specific allergens but also by nonimmunological stimuli such as cold air, exercise, mental stress and infections. However, there have been few in vivo nonimmunological asthma models available as contrasted to the availability of allergic asthma models.

A23187 has been widely used in in vitro experiments as a nonimmunological stimulus to release chemical mediators such as histamine and leukotrienes from mast cells (1-4) in order to screen anti-allergic agents and to study the mechanisms of release of chemical mediators from mast cells. Application of A23187 in in vivo studies of the respiratory tract system has been, however, poorly documented, and there have been no reproducible observations on quantitative airway response.

Aerosolized doses of A23187 at concentrations of 0.00002-0.02% caused no airway responses in rhesus monkeys (8). Dose-related bronchoconstriction and decrease in systemic pressure was observed by i.v. injection of A23187 at doses of 50 and 100 μg in cats (9). Stengel et al. reported that exposure of conscious guinea pigs to A23187 aerosol produced an increase in excised lung gas volumes (10). In this report, they used postmortem pulmonary gas trapping as the index of airway response, and the airway response was obtained only at extremely high concentrations of 0.2-0.8%.

In the present study, we used our inhalation technique to develop a bronchial asthma model using A23187, because bronchial asthma attacks in humans are usually provoked by inhalations of allergens, dust, cold air, and so on. Remarkable asthmatic bronchoconstriction occurred by inhalation of A23187 at concentrations of 0.001-0.005% in guinea pigs. The bronchoconstrictive response had a characteristic pattern: the airway response gradually increased, and 20-25 min was required to reach the maximal response. This is consistent with the results of in vitro experiments (3, 5) using guinea pig lungs which indicate that the releases of histamine and leukotrienes induced by A-23187 were much slower than those induced by antigen.

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**Fig. 2. Effect of chlorpheniramine and FPL-55712 on the change in ventilation overflow induced by inhalation of calcium ionophore A23187 (0.0025% for 5 min).** Each point represents the mean with S.E. from 9 (control), 6 (chlorpheniramine) and 5 (FPL-55712) experiments. *P<0.05, **P<0.01 and ***P<0.001 vs. control group.
The A23187-induced bronchoconstriction was strongly inhibited by chlorpheniramine and FPL-55712. The same doses of chlorpheniramine and FPL-55712 almost abolished the histamine- and leukotriene-induced bronchoconstrictions, respectively. These results suggest that the chief mediators involved in the A23187-induced bronchoconstriction in the present study would be histamine and peptidoleukotrienes. As the effect of the combination of chlorpheniramine and FPL-55712 was not examined in the present study, the possibility of involvement of mediators other than histamine and peptidoleukotrienes is still unknown.

It is expected that this A23187-induced bronchial asthma model in guinea pigs will contribute to studies on antiallergic drugs and airway mast cells.

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