Reversing Effect of Anti-Asthmatic Drugs on Bronchoconstriction Induced by Antigen Challenge and Histamine in Anesthetized Guinea Pigs

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ABSTRACT — We performed an in vivo evaluation of bronchodilation using a model of antigen-induced bronchoconstriction in anesthetized guinea pigs pretreated with indomethacin, pyrilamine and propranolol, and the results were compared with those for the histamine-induced response. Test drugs were administered intravenously when the antigen or histamine response reached its peak. Leukotriene (LT) D₄ antagonists, FPL55712 and LY171883, gradually reduced the antigen-induced response, whereas the lipoxygenase inhibitor phenidone had no such effect. The bronchodilator theophylline rapidly reduced the antigen-induced response, and the adenylate cyclase activator forskolin had a similar effect. The following drugs also had no effect: nifedipine (calcium-channel antagonist), cromakalim (potassium-channel opener), amlexanox and disodium cromoglycate: DSCG (anti-allergic drugs), OKY-046 (thromboxane A₂ synthetase inhibitor), and dapsone (anti-inflammatory drug). Theophylline, the beta-adrenoceptor agonist salbutamol and the histamine H₁-blocker pyrilamine had only a small reversing effect on histamine-induced bronchoconstriction. These results suggest that antigen-, but not histamine-, induced bronchoconstriction in anesthetized guinea pigs is a useful in vivo model for evaluating the bronchodilating effect of anti-asthmatic drugs.

Bronchodilators, theophylline and beta-adrenoceptor agonists are widely used in the therapy of asthma (1, 2). Theophylline inhibits antigen- and agonists-induced bronchospasm in guinea pigs (3, 4), and it also has an anti-allergic effect in rats (5, 6). Similarly, beta-adrenoceptor agonists, salbutamol and isoproterenol, and the adenylate cyclase activator forskolin have anti-asthmatic and anti-allergic activity (7, 8). Calcium-channel antagonists (9, 10) and potassium channel openers (11 – 13) also exhibit bronchodilating effects in the guinea pig, and anti-allergic drugs, DSCG and amlexanox (14), and the TXA₂ synthetase inhibitor OKY-046 (15) inhibit antigen-induced bronchospasm in the guinea pig. However, the bronchodilating effect of these drugs on bronchoconstriction in vivo have not been investigated in detail.

The purpose of the present study was to develop an in vivo system for examining the bronchodilating effect of anti-asthmatic drugs. Therefore, we examined the reversing effects of anti-asthmatic drugs on antigen-induced bronchoconstriction in anesthetized guinea pigs, because antigen causes slowly developing and sustained bronchoconstriction in sensitized guinea pigs pretreated with indomethacin,
pyrilamine and propranolol in contrast to the histamine-induced response. The results of this study demonstrate that antigen-induced bronchoconstriction is a useful in vivo model for examining the abilities of various drugs to reverse this response.

MATERIALS AND METHODS

Materials

Amlexanox, FPL55712, LY171883, OKY-046 and cromakalim were synthesized in our own laboratory. Dapsone, forskolin, indomethacin, nifedipine, phenidone, propranolol hydrochloride, pyrilamine malate, salbutamol, ovalbumin (OA) and urethane were purchased from Sigma Chemical Co., MO, USA. Theophylline was from Nacalai Tesque, Inc., Japan; and DSCG was from Nihon Bulk, Japan. The rabbit anti-chicken egg albumin serum was from Cooper Biomedical, PA, USA; The 4-hr passive cutaneous anaphylaxis (PCA) titer of this antiserum was 1:4000 in guinea pigs and no 7-day PCA activity was observed, suggesting that this antiserum is IgG-rich. Cromakalim, dapsone, forskolin, indomethacin and nifedipine were dissolved in 100% polyethylene glycol (PEG) 400. FPL55712 was dissolved in 0.3% Tween 80 and LY171883, in 8.4% NaHCO₃. Amlexanox was dissolved in 1 N NaOH and diluted with 0.9% saline. Theophylline was dissolved in phosphate-buffered saline (pH 7.4). The other agents were dissolved in 0.9% saline.

Measurement of air overflow

Male Hartley guinea pigs (300 – 400 g) were passively sensitized with rabbit anti-chicken egg albumin (0.125 ml/body, i.v.). One day later, the sensitized animals were anesthetized with urethane (1.5 g/kg, i.p.). The right jugular vein was cannulated for the i.v.-injection. A tracheal cannula was inserted to permit measurement of air overflow by the modified method described by Konzett and Rossler (16). Respiration was carried out as follows: 15 – 20 ml/kg/stroke, 40 – 50 strokes/min. The increase in respiratory overflow volume provoked by OA-challenge or histamine was represented as a percentage of the maximal overflow volume (100%) obtained by clamping off the trachea.

Antigen-induced bronchoconstriction

Following surgical preparation, bronchoconstriction was induced by intravenously administered OA (0.2 mg/kg) in the animals pre-treated with indomethacin (2 mg/kg, 10 min), pyrilamine (2 mg/kg, 6 min) and propranolol (0.1 mg/kg, 5 min) before OA challenge (0.2 mg/kg) to eliminate any contribution by endogenous prostaglandins, histamine and catecholamines.

Histamine-induced bronchoconstriction

Bronchoconstriction was induced by intravenous injection of histamine (10 μg/kg).

Reversal of bronchoconstriction

The peak bronchoconstriction induced by antigen or histamine in the approximately 50 – 70% range was used in these experiments. When the response reached a peak level, drugs or vehicles were administered intravenously and the reversing effect was measured for 10 min (OA) or 5 min (histamine). Maximal reversal by anti-asthmatic drugs was calculated as follows: % maximal reversal = [% peak bronchoconstriction before the injection of drug or vehicle – % bronchoconstriction at maximal reversal] / [% peak bronchoconstriction before the injection of drug or vehicle] × 100

Prophylactic effect of drugs on bronchoconstriction

Drugs were administered intravenously 1 min before antigen challenge. The percent inhibition of drugs was calculated as follows: % Inhibition = [% peak bronchoconstriction of control groups – % peak bronchoconstriction of drug-treated groups] / [% peak bronchoconstriction of control groups] × 100

Statistics

Results are expressed as means ± S.E.M.
Statistical significance (P < 0.05) of the data was evaluated by Student's t-test for unpaired data.

RESULTS

Antigen-induced bronchoconstriction

Intravenous injection of OA into anesthetized guinea pigs caused slowly developing and sustained bronchoconstriction with a peak response in 5–10 min. LT antagonists, FPL55712 (5 mg/kg) and LY171883 (5 and 10 mg/kg) intravenously administered at the time of the peak response reduced gradually but significantly the antigen-induced response, whereas the lipoxygenase inhibitor phenidone (10 mg/kg), which prevented the antigen-induced response when administered 1 min before OA challenge (Table 2), had no effect on it (Fig. 1 and Table 1). Theophylline (50 mg/kg) and forskolin (0.1 mg/kg) produced rapid and significant reduction of antigen-induced bronchoconstriction (Fig. 2 and Table 3). Nifedipine (0.1 mg/kg), cromakalim (1 mg/kg), dapsone (30 mg/kg), DSCG (20 mg/kg), OKY-046 (5 mg/kg) and amlexanox (10 mg/kg) had no effect (Table 4), while nifedipine, cromakalim,

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<th>Table 1. Effect of LT antagonists and a lipoxygenase inhibitor on reversal of antigen-induced bronchoconstriction</th>
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<td>0.3% Tween 80</td>
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<td>FPL55712</td>
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<td>8.4% NaHCO₃</td>
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Drugs (i.v.) were administered when the bronchoconstriction reached its peak. Results are shown as the means ± S.E.M. *P < 0.05, significantly different from the vehicle control.

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<td>Amlexanox</td>
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Drugs (i.v.) were administered 1 min before the antigen challenge. Results are shown as the means ± S.E.M. *P < 0.05, significantly different from the vehicle control.

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<th>Table 3. Reversal of antigen-induced bronchoconstriction by theophylline and forskolin</th>
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<td>Saline</td>
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dapsone, amlexanox, but not DSCG, were effective when administered 1 min before OA challenge (Table 2).

**Histamine-induced bronchoconstriction**

Histamine (10 μg/kg) induced a transient bronchoconstriction with a peak at 10–20 sec, which was followed by a decline. Theophylline (50 mg/kg), salbutamol (0.5 mg/kg) and pyrilamine (1 mg/kg) had little reversing effect on the histamine response (Fig. 3). The dose of pyrilamine used in this experiment completely prevented histamine induced bronchoconstriction when the animals were treated with the

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drug before the histamine challenge (data not shown).

DISCUSSION

Although there is no perfect model for bronchial asthma, animal models are needed to study anti-asthmatic drugs. Guinea pigs have been used in experimental models for asthma, since their airway reactivities to bronchoconstrictors and the mediators released in allergic reactions are similar to those found in asthmatic patients (17, 18). Slow reacting substance of anaphylaxis (SRS-A), which consists of peptide-LTs, is an important mediator of pulmonary anaphylaxis in humans and guinea pigs (19). In sensitized guinea pigs pretreated with indomethacin, pyrilamine and propranolol, antigen challenge causes slowly developing and sustained bronchoconstriction indicative of a SRS-A response. This response is prevented by LT antagonists and lipoxygenase inhibitors (20, 21, this paper). In the present study, the LT antagonists FPL55712 and LY171883 gradually reversed antigen-induced bronchoconstriction, but the lipoxygenase inhibitor phenindone, which was prophylactically effective, did not have any significant effect. These results suggest that antigen-induced bronchoconstriction is already established when it reaches a peak. This interpretation is also supported by our data that the mediator release inhibitor amlexanox, which was prophylactically effective and has been reported to exhibit prophylactic effects on experimental bronchoconstriction (22), had no reversing effect on antigen-induced bronchoconstriction. In the present study, theophylline and the direct adenylate cyclase activator forskolin rapidly reversed the antigen-induced response. In contrast, the calcium-channel antagonist nifedipine and potassium-channel opener cromakalim, which were prophylactically effective (this paper) and have bronchodilating activity (9–13), had no effect on it. Though we do not know why nifedipine and cromakalim do not exhibit the reversing effect under our experimental conditions, this evaluation system is a useful in vivo model for bronchodilation, because theophylline, which has been widely used in asthmatic patients, produces a rapid reversal of antigen-induced bronchoconstriction in anesthetized guinea pigs.

Dapsone has been used as an anti-inflammatory drug (23). This compound inhibits the arachidonate lipooxygenase pathway and LTB4 binding (24). In the present study, dapsone had no reversing effect on the antigen-induced response. In contrast, when given prophylactically at the same dose before antigen chal-
lenge, dapsone significantly protected the animals against antigen-induced bronchoconstriction. These observations suggest that the prophylactic effect of dapsone on the antigen-induced response involves lipoxygenase inhibition, similar to phenidone, indicating that LTB4 may not contribute to the antigen-induced response.

Histamine, as well as SRS-A, is a very important chemical mediator of allergic bronchoconstriction in both humans and guinea pigs (1). However, airway response induced by endogenous SRS-A is long-lasting, while that induced by exogenous histamine is transient. Antigen-induced histamine-mediated bronchoconstriction is also known to be transient (25). In this study, neither theophylline, salbutamol nor the histamine H1 antagonist pyrilamine reversed histamine-induced bronchoconstriction. The reason for the failure of pyrilamine to antagonize the histamine response is not clear, but may be related to the possibility that the intracellular mechanism of bronchoconstriction induced by exogenously administered histamine is different from that by endogenously generated SRS-A under these experimental conditions.

In conclusion, we examined the reversing effect of anti-asthmatic drugs on antigen- and histamine-induced bronchoconstriction in the guinea pig. The clinically effective bronchodilators used markedly reversed the antigen-induced response, but had no effect on the histamine response. These results suggest that the evaluation of reversal by using antigen-induced bronchoconstriction in the guinea pig is feasible for studying in vivo bronchodilation by anti-asthmatic drugs.

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