The Equivalent Bronchodilator Effects of Salbutamol Formulated in Chlorofluorocarbon and Hydrofluoroalkane-134a Metered Dose Inhalers on the Histamine-Induced Pulmonary Response in Dogs

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Purpose. The bronchodilator effect of salbutamol formulated in hydrofluoroalkane-134a (HFA-134a), a chlorofluorocarbon (CFC)-free propellant for metered dose inhalation (MDI) devices, was compared with that of salbutamol formulated in CFC in anesthetized dogs.

Methods. Bronchospasms were induced by the intravenous injection of histamine, and bronchial resistance was measured by the method of Konzett and Rossler.

Results. While the placebo vehicles (HFA-134a and CFC propellants) had no significant effect on histamine-induced bronchospasms, the salbutamol/HFA-134a and salbutamol/CFC MDI formulations had equivalent dose-related inhibitory effects.

Conclusions. These data indicated that salbutamol formulated in HFA-134a and that in CFC propellant are bioequivalent.

KEY WORDS: bioequivalent; salbutamol; hydrofluoroalkane-134a (HFA-134a); Konzett-Rossler.

INTRODUCTION

Adrenergic β2-agonists such as salbutamol are widely used for the treatment of reversible airway obstruction. The use of metered dose inhalers to deliver drugs directly to the respiratory system has been established as an accepted, reliable and effective procedure for the treatment of asthmatic symptoms. Chlorofluorocarbons (CFCs) are currently used as propellants for metered dose inhalers. However, it is well known that chlorine is released from CFCs on their exposure to ultraviolet rays and thus they destroy the ozone layer in the earth's upper atmosphere. For this reason, the continued use of CFC is being restricted under the conditions of the Montreal Protocol (1987) (1). A chlorine-free hydrofluoroalkane (HFA) propellant such as 1,1,1,2-tetrafluoroethane has been considered as an alternative among other propellants. In addition, clinical studies comparing metered dose inhalation (MDI)-delivered drugs, formulated in CFC and HFA propellants, demonstrated similar efficacy (2–4). The current study extended these previous studies and compared the effects of MDI-delivered salbutamol, formulated in a CFC propellant (CFC-11,12) or in an HFA-134a propellant, on histamine-induced bronchoconstriction in anesthetized dogs, using the Konzett-Rossler method (5–7), a well established pulmonary pharmacological model. Additionally, blood pressure and heart rate were monitored continuously throughout the experiment.

MATERIALS AND METHODS

Animals

Male beagle dogs (Yakken Farm, Hyogo, Japan) weighing 9–12 kg were used. They were housed with free access to food (Oriental Yeast Co., Ltd., Japan) and water in a room maintained at 23 ± 5°C and 55 ± 10% humidity under a 12-hr light-dark cycle.

Materials

Salbutamol, a β_2-adrenergic agonist, was formulated with a chlorofluorocarbon (CFC) propellant, Glaxo's Sultanol®, or with a hydrofluoroalkane-134a (HFA-134a) propellant, 3M's Airomir®. Sultanol® and Airomir® are referred to as salbutamol/CFC and salbutamol/HFA-134a, respectively, in this paper. The Salbutamol/HFA-134a 200 dose and 100 dose formulations were provided by 3M Pharmaceuticals (St. Paul, MN). Salbutamol/CFC was purchased from Glaxo Co. The CFC and HFA-134a propellants were supplied by 3M Pharmaceuticals. The agents used as bronchoconstrictors were histamine dihydrochloride (Nacalai Tesque, Kyoto, Japan), methacholine chloride (Nacalai Tesque, Kyoto, Japan), acetylcholine chloride (Nacalai Tesque, Kyoto, Japan), and leukotriene D_4 (Cascade Biochem. Ltd., BK.). They were dissolved in saline and diluted to appropriate concentrations with saline.

Konzett-Rossler Method for Measuring Bronchoconstriction

Adult male beagle dogs were given a tranquilizer (propyonyl promazine, 7 mg/dog) 30 minutes prior to anesthesia by intravenous injection of 30 mg/kg of sodium pentobarbital. During the experiment, anesthesia was maintained by intravenous infusion of sodium pentobarbital at the rate of 4 mg/kg/hr, using a pump via a cannula inserted into the left femoral vein. Bronchial resistance was measured by the modified Konzett-Rossler method (5). The trachea was cannulated with a Y shaped glass cannula with two branches, and respiration was maintained with a respirator (Model SN-480-7; Shinano-Kogyo Co., Ltd., Tokyo, Japan) at the rate of 18 breaths/minute, with an air volume of 18 to 22 ml/kg. Respiratory overflow was measured with a differential pressure transducer via one branch of the cannula. The test agents were delivered by aerosol inhalation exposure to the trachea via the other branch of the cannula, synchronously with the respiratory cycle. The MDI dose delivered into the cannula was 100 μg per puff. The challenge agents were delivered via a cannula inserted into the right femoral vein. Bronchoconstrictor responses were represented as the increased excursions of the tracing on a physiological recorder. At the end of the experiment, each dog was given a high dose of the bronchoconstrictor and the maximal response was measured in order to calculate the percentage to maximal occlusion. The blood pressure and heart rate were also monitored continuously

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throughout the experiment. The arterial blood pressure was measured in the right femoral artery with a cannula connected to a pressure transducer, and the heart rate was determined from the arterial pulses with a tachometer.

Experimental Design

Study 1

(Selection of a challenge agent): Histamine (1, 2, 4, 8, 16 and 32 μg/kg), methacholine (0.5, 1, 2, 4 and 8 μg/kg), acetylcholine (1, 2, 4, 8, 16 and 32 μg/kg) and leukotriene D4 (0.1, 0.2, 0.4, 0.8 and 1.6 μg/kg) were successively injected intravenously as challenge agents at 15 minute intervals. Six dogs were used for this study, and each dog was given all the challenge agents in a different order of administration.

Study 2

(Bioequivalence of salbutamol/HFA-134a and salbutamol/CFC). The inhibitory effect on the bronchial response to histamine of salbutamol/HFA-134a was compared with that of salbutamol/CFC.

Four to five dogs were exposed to 1 puff of actuated aerosol administration from a metered dose inhalation device containing salbutamol/CFC, and then were challenged with 8 μg/kg of histamine six times at 5, 15, 30, 60, 90 and 120 minutes after the aerosol administration. After an hour's rest, the dogs were exposed to 1 puff of the salbutamol/HFA-134a 200 dose formulation and then challenged with histamine six times again. An additional group consisting of 4 to 5 dogs was treated in the same manner as above but with the reverse order of administration of the aerosols. Eight additional groups consisting of 4 to 5 dogs were treated with 2 puffs/4 puffs of the 200 dose formulation, and 1 puff or 4 puffs of the 100 dose formulation. One dog was used for an experiment in one day. An additional 8 to 10 dogs were subjected to aerosol administration of the vehicles and were examined for the bronchial response to histamine in the same manner as above.

Statistical Analysis

Data were summarized and expressed as means ± standard error of the mean (S.E.M.) for changes in the ventilation overflow volume (ml), % changes in the volume (ml), % inhibition by the drugs, and % of the maximal occlusion response to the histamine challenge. The statistical significance of the differences between means was determined by repeated measure ANOVA. In addition, one-way ANOVA was performed for summarized values for each treatment/time point combination. In the case of blood pressure and heart rate, one-way ANOVA was performed.

RESULTS

Study 1: Selection of a Challenge Agent

Transient increases in bronchial resistance were induced by intravenous injections of histamine, methacholine and acetylcholine, but not leukotriene D_4 (Fig. 1). Histamine (8 μg/kg) was chosen as a bronchoconstrictor stimulant because it gave a moderate response of 20–30% of the maximal occlusion.

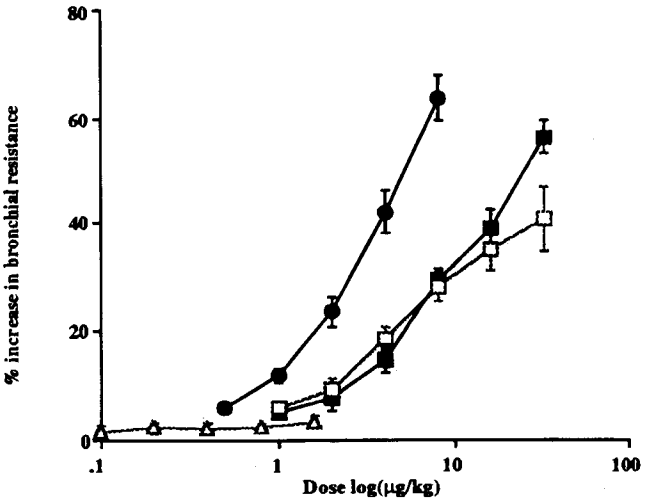

Fig. 1. Bronchoconstriction in response to increasing doses of histamine (■), acetylcholine (□), methacholine (●), and leukotriene D_4 (△) in dogs. Vertical bars show the S.E.M. N = 6 dogs.

Repeated administration of 8 μg/kg of histamine to dogs elicited the same level of bronchoconstrictive response each time for at least 5–6 hours (data not shown).

Study 2: Bioequivalence of Salbutamol/HFA-134a and Salbutamol/CFC

The inhibitory effects on histamine-induced bronchoconstriction of salbutamol/CFC and salbutamol/HFA-134a were examined. As shown in Fig. 2, both drugs inhibited the response in a good dose-related manner, while no inhibitory effects of the propellant vehicles, HFA-134a and CFC, were observed. Next, the level of inhibitory effect was compared between the salbutamol/CFC treated and salbutamol/HFA-134a treated groups. In Fig. 3, histamine-induced bronchoconstriction after the administration of 1 puff or 4 puffs of the salbutamol/HFA-134a 200 dose formulation or salbutamol/CFC formulation is shown. No statistical difference in the inhibitory effect between the two drugs was observed at any time point. Moreover, no difference in the inhibitory effect was observed between the groups administered 2 puffs of the salbutamol/HFA-134a 200 dose formulation and the salbutamol/CFC formulation, or between the groups administered 1 puff or 4 puffs of the salbutamol/HFA-134a 100 dose formulation and the salbutamol/CFC formulation (data not shown). Furthermore, no changes in basal resistance, as measured as the overflow amount, were observed after MDI administration of salbutamol/CFC or salbutamol/HFA-134a (data not shown).

Influence on the Blood Pressure and Heart Rate of Dogs

The intravenous administration of histamine (8 μg/kg) caused decreases in systolic blood pressure of about 60 mmHg and in diastolic blood pressure of about 50 mmHg, and also caused a decrease in the heart rate of 10 beats/minute. The influence of salbutamol/CFC or salbutamol/HFA-134a on the blood pressure and heart rate was examined. As shown in Fig. 4, 4 puffs administration of the salbutamol/HFA-134a 200 dose formulation and the salbutamol/CFC formulation did not affect the cardiovascular system. Furthermore, at other doses, they

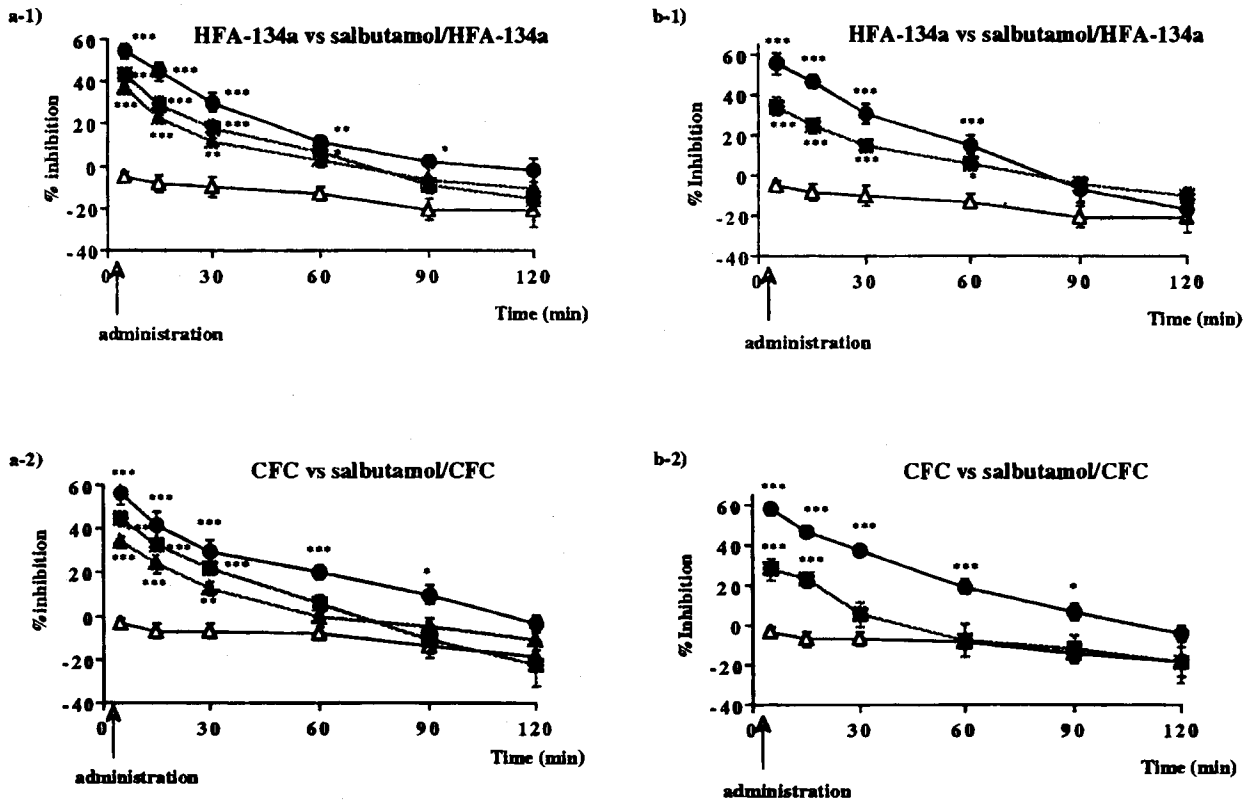

Fig. 2. Inhibitory effects of salbutamol/CFC and salbutamol/HFA-134a (100 dose or 200 dose formulation) on bronchoconstrictor responses induced by histamine in dogs.

Each point represents the mean percent inhibition of percent maximum occlusion in the bronchoconstrictor responses to histamine. Panel a-1) Salbutamol/HFA-134a 200 dose formulation; 1 puff (▲), 2 puffs (■), 4 puffs (●), and HFA-134a placebo (△). Panel a-2) Salbutamol/CFC; 1 puff (▲), 2 puffs (■), 4 puffs (●), and CFC placebo (△). Panel b-1) Salbutamol/HFA-134a 100 dose formulation; 1 puff (▲), 4 puffs (●), and HFA-134a placebo (△). Panel b-2) Salbutamol/CFC 100 dose formulation; 1 puff (▲), 4 puffs (●), and CFC placebo (△). Vertical bars show the S.E.M. N = 8 ~ 10 dogs. Significant differences from the placebo-treated group are shown as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

did not affect the cardiovascular system (data not shown). In all cases, no change in the basal blood pressure level or the heart rate was observed. Moreover, no remarkable influence on the decrease in blood pressure was observed after administration of the MDI propellant vehicles.

DISCUSSION

In the present studies, salbutamol formulated in HFA-134a was compared with that formulated in CFC as to the ability to inhibit histamine-induced bronchoconstriction in anesthetized dogs. Pulmonary responses were monitored using the air overflow method of Konzett and Rossler. The advantage of experiments involving dogs is that not only the bronchodilator activity, but also the systemic influences such as cardiovascular events, can be monitored simultaneously in one dog on the same day. In contrast, in human studies, the efficacy of drugs and their adverse effect on the cardiovascular system are examined in individual clinical studies (2–4).

In order to establish an evaluation method for metered dose inhalers, we first examined the bronchoconstrictive responses to various spasmogens to select suitable conditions. Chemical mediators such as histamine, acetylcholine and leukotriene D_4 are well known to be involved in the pathophysiology of asthma. Histamine, acetylcholine and methacholine were shown to induce a transient increase in bronchial resistance in a good dose-related manner, whereas leukotriene D_4 induced only very weak bronchoconstriction in dogs (Fig. 1). Our results for leukotriene D_4 were consistent with those of Daniel *et al.* (8), i.e., leukotriene D_4 exhibited low efficacy in canine airways, which was proposed to be due to the high PGE_2 relaxant tone in this species. The present study was designed to evaluate the biological equivalence by means of a 'crossover' method, i.e., by comparing the efficacy of the drugs in the same animal, because the bronchial response to histamine was reproducible in the same animal, at least within 5–6 hours.

As shown in Fig. 2, the inhibitory effect of salbutamol in HFA-134a or CFC continued for a fairly long period. In most cases, the inhibitory effect was significant until 60 minutes after the administration, but no longer observed at 120 minutes. The long duration of the action of small doses of salbutamol administered as an aerosol suggests that the drug is slowly mobilized from the lungs. In fact, 100–200 μg of the drug administered as an aerosol acted for longer periods than did 4–10 mg of the drug given orally (6). The statistical studies suggested that salbutamol formulated in HFA-134a and that in CFC propellant are equivalent in the inhibition of histamine-

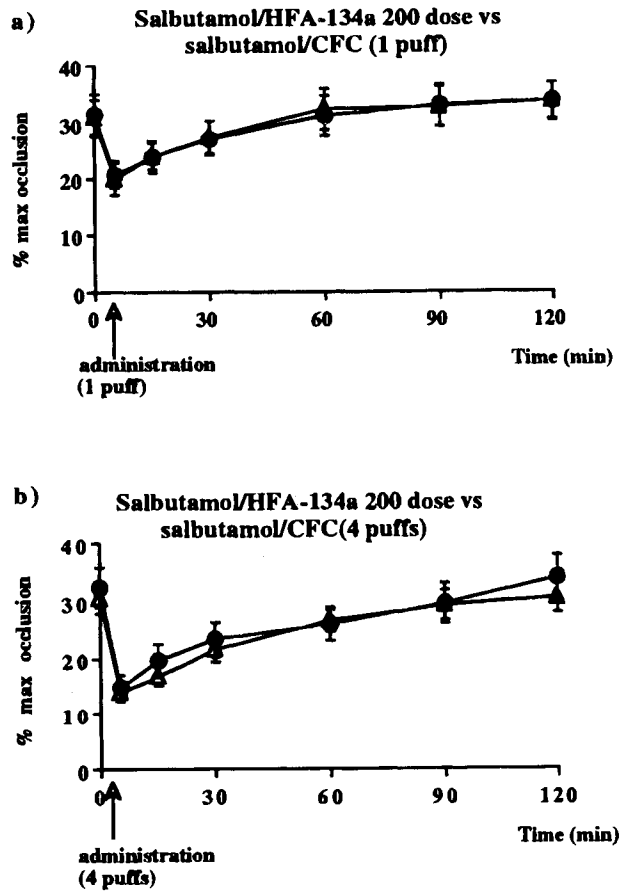

Fig. 3. Comparison of the inhibitory effects of salbutamol/CFC and salbutamol/HFA-134a on bronchoconstrictor responses induced by histamine in dogs.

Panel a) Salbutamol/HFA-134a vs salbutamol/CFC, 1 puff of 200 dose formulation. Panel b) Salbutamol/HFA-134a vs salbutamol/CFC, 4 puffs of 200 dose formulation. Each point represents the mean percentage of the maximum occlusion in the bronchoconstriction responses to histamine. Open triangles and closed circles represent the salbutamol/HFA-134a treated and salbutamol/CFC treated groups, respectively. Vertical bars represent S.E.M. N = 8 ~ 10 dogs. No significant differences exist.

induced bronchoconstriction in dogs. The minimum detectable difference in this analysis might be 10%, in terms of bronchial resistance.

In addition to the inhibitory effect on histamine-induced bronchoconstriction, the influence of inhaled salbutamol on the cardiovascular system was studied in order to determine whether or not this β_2 agonist, delivered in either HFA-134a or CFC, had different effects on non-pulmonary systems. The HFA-134a and CFC formulated salbutamol products and their propellant vehicles did not affect the histamine-induced decrease in blood pressure. As a result, this study demonstrated the lack of a difference between the effects of salbutamol/HFA-134a and salbutamol/CFC on the cardiovascular system.

In conclusion, the present studies suggest that the salbutamol/HFA-134a formulation showed equivalent efficacy to the salbutamol/CFC formulation. In addition, HFA-134a had no influence on histamine-induced bronchoconstriction, blood pressure or heart rate in anesthetized dogs. Therefore, these results further confirm that HFA-134a may be considered a suitable alternative propellant for metered dose inhalers.

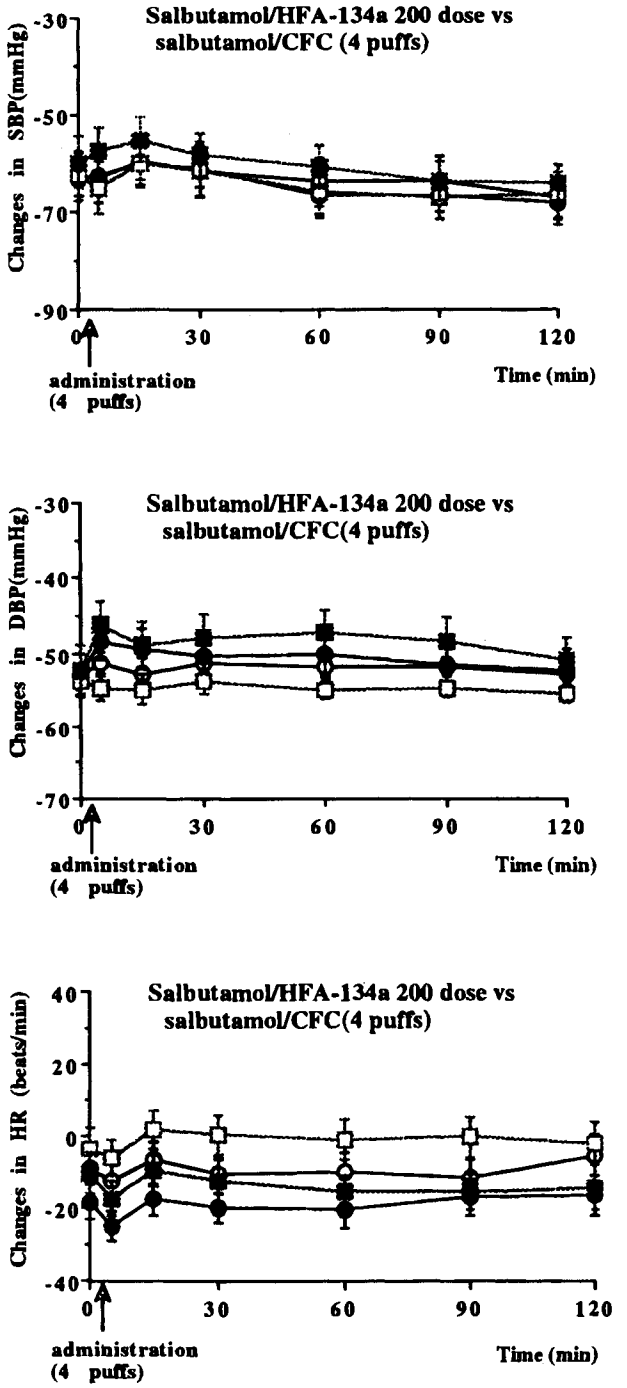

Fig. 4. Influence of salbutamol/CFC and salbutamol/HFA-134a (4 puffs of 200 dose formulation) on the changes in systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) caused by histamine administration in anesthetized dogs.

Open circles and squares represent the HFA-134a and CFC treated groups, and closed circles and squares the salbutamol/HFA-134a and salbutamol/CFC treated groups, respectively. Each point represents the mean value of the decrease in blood pressure or heart rate induced by histamine injection. Vertical bars represent S.E.M. N = 8 ~ 10 dogs.

REFERENCES

1. Montreal Protocol. Sept. 16, 1987, 26 ILM 1541 (1987).
2. E. R. Bleecker, N. M. Klinger, B. P. Ekholm, and D. G. Tinkelman. *Am. J. Respir. Crit. Care Med.* **151**:A58 (1995).
3. M. Jenkins. *J. Aerosol Med.* **8**:541–547 (1995).
4. R. Ruffin, C. Mitchell, P. Thompson, D. Harle, A. Cline, and B. P. Ekholm. *Am. J. Respir. Crit. Care Med.* **151**:A58 (1995).
5. H. Konzett, and R. Rossler. *Arch. Exp. Path. Pharmak.* **195**:71–74 (1940).
6. V. A. Cullum, J. B. Farmer, D. Jack and G. P. Levy. Br. *J. Pharmacol.* **35**:141–151 (1969).
7. Y. Yabuuchi, S. Yamashita, and S. Tei. *J. Pharmacol. Exp. Ther.* **202**:326–336 (1977).
8. E. E. Daniel, A. P. Abela, L. J. Janssen, and P. M. O'Byrne. *Can. J. Physiol. Pharmacol.* **70**:624–634 (1992).