Br. J. Pharmacol. (1993), **108**, 1016–1023

Enhancement of airway reactivity to histamine by isoprenaline and related β-adrenoceptor agonists in the guinea-pig

Barbara C. Galland & [1]J.G. Blackman

Department of Pharmacology, Otago Medical School, University of Otago, P.O. Box 913, Dunedin, New Zealand

1 The effect of isoprenaline, adrenaline and salbutamol on airway reactivity to histamine was observed in anaesthetized, ventilated guinea-pigs. Airway reactivity was determined before and 20 min and 90 min after a 30-min i.v. infusion of each agonist by constructing cumulative dose-response curves from breath-by-breath measurements of the effect of different rates of i.v. infusion of histamine on lung resistance (R_L) and dynamic compliance (C_{dyn}).

2 (±)-Isoprenaline infused i.v. for 30 min at a rate of 0.4 μmol h^{-1} kg^{-1} caused bronchodilatation and a fall in blood pressure. Recovery to starting values of R_L and C_{dyn} occurred within 20 min of stopping the infusion.

3 Reactivity to histamine was greatly enhanced when measured 20 min and 90 min after stopping the infusion of (±)-isoprenaline. This was not an effect of the prior infusion of histamine or of the dissolving solution.

4 Infusion of (−)-isoprenaline for 30 min at a rate of 0.2 μmol h^{-1} kg^{-1} also enhanced reactivity to histamine. However, enhancement of reactivity to histamine was not demonstrable after infusion of (+)-isoprenaline at equal or higher dose rates.

5 Infusions of bronchodilator concentrations of adrenaline and salbutamol also enhanced airway reactivity to histamine, but the bronchodilator effect of salbutamol lasted longer than that of isoprenaline or adrenaline and the development of hyperreactivity was delayed.

6 After acute bilateral vagotomy, infusion of (±)-isoprenaline enhanced airway reactivity but only at the highest dose of histamine.

7 (±)-Isoprenaline did not enhance contractile responses to histamine in isolated preparations of first branch bronchi.

8 We conclude that the bronchodilator effect of activating β-adrenoceptors in the airways of guinea-pigs is followed by a more persistent state of hyperreactivity to histamine.

Keywords: β-Agonists; isoprenaline; adrenaline; salbutamol; histamine; airway hyperreactivity; lung resistance, isolated bronchi

Introduction

A number of investigators have reported indirect evidence of enhanced reactivity to bronchoconstrictor stimuli in animals treated with isoprenaline and related agonists. Thus, Conolly *et al.* (1971) noted increased mortality from histamine-induced bronchospasm in guinea-pigs pretreated with i.m. isoprenaline, terbutaline or salbutamol and Bouhuys *et al.* (1972) reported that i.m. isoprenaline increased mortality to histamine given i.p. to guinea-pigs. Also, Izard *et al.* (1971) found that mortality from antigen given i.v. to sensitized guinea-pigs was increased after treatment with i.p. adrenaline.

Recently, Morley and co-workers (Mazzoni *et al.*, 1987; Morley & Sanjar, 1987; Sanjar *et al.*, 1990) have reported that an i.v. infusion of (±)-isoprenaline in the anaesthetized guinea-pig enhanced the bronchoconstrictor effect of a fixed dose of bombesin, an effect prevented by bilateral vagotomy. They also reported that the (+)-isomer, which at similar concentrations lacks β-adrenoceptor agonist activity, was as effective as the racemate. Noradrenaline and dopamine also enhanced responses to bombesin, but adrenaline did not. Most recently, Morley *et al.* (1991) have presented evidence that the two optical isomers of salbutamol also enhance airway reactivity in the guinea-pig. The authors conclude that these compounds induce airway hyperreactivity by a mechanism not involving stereoselective activation of β-adrenoceptors but dependent on an intact vagal innervation. Since an important aim of treatment of asthma is to reduce the airway hyperreactivity characteristic of the disease, these unexpected findings are both cause for concern and worthy of further study, for they provide an experimental basis for clinical reports (as discussed later) that β-agonist bronchodilators can enhance airway reactivity in asthmatic patients and make control of their symptoms more difficult.

Prompted by the original reports of Morley and co-workers, we explored this effect of β-agonists on airway reactivity in the anaesthetized guinea-pig by using cumulative dose-response curves rather than responses to single doses of the bronchoconstrictor agent as a measure of reactivity. Our results, derived from breath-by-breath measurements of lung resistance and dynamic compliance, confirm the primary observation of Morley and co-workers, but differ in some other respects. Preliminary accounts of this work have been published (Galland & Blackman, 1988; 1989).

Methods

For the experiments *in vivo*, guinea-pigs (weight 467 ± 8 g, mean ± s.e.mean, $n = 42$) were anaesthetized with pentobarbitone (40 mg kg^{-1}, i.p.), paralyzed with suxamethonium (0.5 mg kg^{-1}, i.v.) and ventilated at 1 Hz through a tracheal cannula with a constant volume positive pressure pump set to deliver 7.0 ml kg^{-1} body wt. Rectal temperature was maintained at 38°C with a thermostatically-controlled heating pad. The chest of each animal was opened and end-expiratory pressure was maintained at 200–250 Pa. The right carotid artery was cannulated for measuring blood pressure and the left jugular vein cannulated for injecting or infusing solutions of drugs.

[1] Author for correspondence.

Measurement of R_L and C_{dyn}^{-1}

Animals were placed in a body plethysmograph and lung resistance (R_L) and the reciprocal of dynamic compliance (C_{dyn}^{-1}) were computed breath-by-breath from transpulmonary pressure, tidal volume and flow rate by the isovolume technique described by Amdur & Mead (1958). Transpulmonary pressure was measured as the difference between the pressure in the side arm of the tracheal cannula and the pressure in the plethysmograph, which, because the chest was open, gave a direct measure of pressure in the pleural space. Tidal volume excursions were recorded by calibrating the pressure change measured in the plethysmograph with a differential pressure transducer. Flow rates were obtained from the slopes of lines fitted to the tidal volume curve at the mid-inspiratory and mid-expiratory points (isovolume points). The on-line computations were performed with a programme written by Dr R.D. Purves for an Apple IIe computer equipped with a 12-bit analogue-to-digital converter (Galland *et al.*, 1986). Baseline values of R_L and C_{dyn}^{-1} were reliably recorded by the method, but during infusion of histamine, particularly at the higher dose rates, occasional irregularities in the tidal volume curve produced discrepant estimates of R_L and C_{dyn}^{-1}. These were readily detected and were deleted before the results were analysed.

Infusion of β-adrenoceptor agonists

Racemic isoprenaline, its (+)- and (−)-isomers, and salbutamol, were dissolved in 0.9% NaCl immediately before infusion into the jugular vein at a rate of 0.05 ml min^{-1} for 30 min with a constant velocity pump. Immediately before infusion of adrenaline at the same rate, the stock solution of adrenaline containing Na metabisulphite (0.1% w/v) was diluted with 0.9% NaCl to the required concentration. The 42 animals used in these experiments were allocated to 8 experimental groups for infusion of the different drugs as follows: (±)-isoprenaline ($n = 4$); (±)-isoprenaline after vagotomy ($n = 6$); (+)-isoprenaline ($n = 7$); (−)-isoprenaline ($n = 5$); saline control ($n = 6$); salbutamol ($n = 4$); adrenaline ($n = 6$); Na metabisulphite control ($n = 4$).

Histamine infusion

As a measure of airway reactivity, dose-response curves to histamine were determined 20 min before, and 20 and 90 min after the 30-min infusion of isoprenaline (or other agonist) by measuring the peak changes in R_L and C_{dyn}^{-1} produced by infusing a fixed concentration of histamine into the jugular vein at increasing rates equivalent to 12, 24 and 48 μg min^{-1} kg^{-1}, and in some experiments equivalent to 96 μg min^{-1} kg^{-1}, each in succession for 90 s. The flow of solution at the lowest infusion rate of histamine was 0.03 ml min^{-1} so that the total volume infused was either 0.6 ml or 1.4 ml delivered over 4.5 or 6 min respectively. Anaesthesia and paralysis were maintained during the 3 h of each experiment with at least 3 supplementary injections of pentobarbitone and suxamethonium made at appropriate intervals.

Vagotomy

For vagotomy, the nerves were identified in 6 guinea-pigs and freed ready for transection at least 20 min before the first determination of airway reactivity to histamine. Both vagus nerves were then cut and 50 min later reactivity was determined again. The solution of (±)-isoprenaline was then infused for 30 min. Twenty min after stopping the infusion, reactivity to histamine was determined a third time.

Isolated bronchial smooth muscle preparations

For the experiments *in vitro*, four guinea-pigs (434 ± 11 g, mean ± s.e.mean) were anaesthetized with pentobarbitone (50 mg kg^{-1}). The trachea and attached lungs were quickly removed and placed in ice-cold Krebs solution of the following composition (mmol l^{-1}): NaCl 120, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.7. Both left and right first branch bronchial rings from each guinea-pig were dissected and prepared for isometric recording according to the method of Hooker *et al.* (1977). Each preparation was mounted in a 50 ml organ bath filled with Krebs solution maintained at 37°C and bubbled with a 95% O_2, 5% CO_2 gas mixture. The resting muscle tension was adjusted to 1 g wt and the tissue allowed to equilibrate for 60 min. During this time the solution was frequently replaced. Each bronchial ring was exposed to cumulative increases in the concentration of histamine given every 4 min until a maximal response was reached. The histamine was then washed out and the tissue allowed to return to baseline tension for 1 h. Racemic isoprenaline (1 μmol l^{-1}) was added to the organ bath for 30 min, washed out, and responses to cumulative increases in the concentration of histamine were again recorded 20 min later. The sensitivity of the bronchial smooth muscle preparations was estimated from the cumulative concentration-response curves as the effective concentration producing 50% of the maximal response (EC_{50}).

Statistical analyses

Results are expressed as mean values ± standard error of the mean (s.e.mean). The significance of differences between dose-response curves was determined by two-way analysis of variance (CLR ANOVA programme for Apple Macintosh). For the *in vitro* experiments, the significance of differences between EC_{50} values was calculated by Student's *t* test.

Drugs

Use in the text of the name of a β-agonist without qualification implies that the (±)-isomer was used. The drugs and suppliers were as follows: histamine diphosphate (Sigma); (±)-isoprenaline hydrochloride (Sigma); (+)-isoprenaline (+)-bitartrate (Sigma); (−)-isoprenaline (+)-bitartrate (Sigma); salbutamol hemisulphate (Sigma); adrenaline hydrogen tartrate (adrenaline injection B.P.); sodium pentobarbitone (Abbott); succinylcholine chloride (Sigma). The identity of the sample of (+)-isoprenaline (+)-bitartrate was confirmed by determination of the specific optical rotation ($[\alpha]_D^{25} = +38°$) and the infra-red spectrum (Keller, 1986).

Results

Time course of bronchodilator effect of i.v. infusions of agonists

Figure 1 shows that an i.v. infusion for 30 min of each of the three β-agonists, isoprenaline, adrenaline and salbutamol caused a progressive fall in lung resistance (R_L) and in the reciprocal of the dynamic compliance (C_{dyn}^{-1}). The bronchodilator response was usually rapid in onset and approached steady-state within 20 to 30 min of starting the infusion. With isoprenaline and adrenaline, recovery on stopping the infusion was rapid: both R_L and C_{dyn}^{-1} approached baseline values within 20 min. With salbutamol, the value of C_{dyn}^{-1} recovered rapidly also (Figure 1b), but the value of R_L remained low for at least 20 min (Figure 1a), recovering, however, within 90 min. In three separate experiments with isoprenaline, we found that the same degree of bronchodilatation was produced by a second infusion 30 min later, indicating that tachyphylaxis did not occur.

Cardiovascular effects of β-agonists and histamine

The i.v. infusion of (±)-isoprenaline, (−)-isoprenaline, adrenaline and salbutamol, all caused arterial blood pressure to

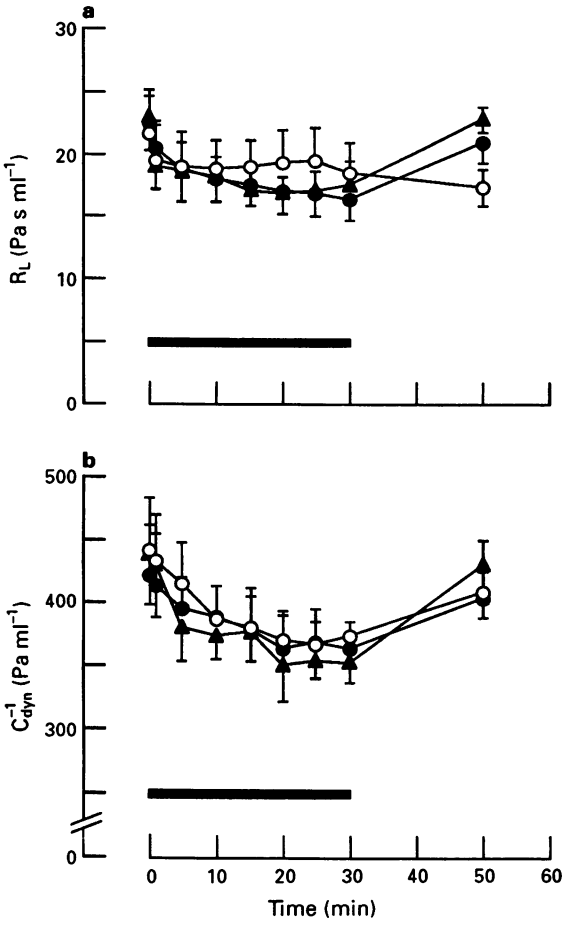

Figure 1 Changes in mean absolute values of R_L (a) and C_{dyn}^{-1} (b) produced by infusions of 0.4 μmol h^{-1} kg^{-1} isoprenaline (●), 0.4 μmol h^{-1} kg^{-1} salbutamol (○), and 0.3 μmol h^{-1} kg^{-1} adrenaline (▲). Data from 4, 4, and 6 guinea-pigs respectively; s.e.mean shown by vertical bars. Horizontal bar shows duration of infusion.

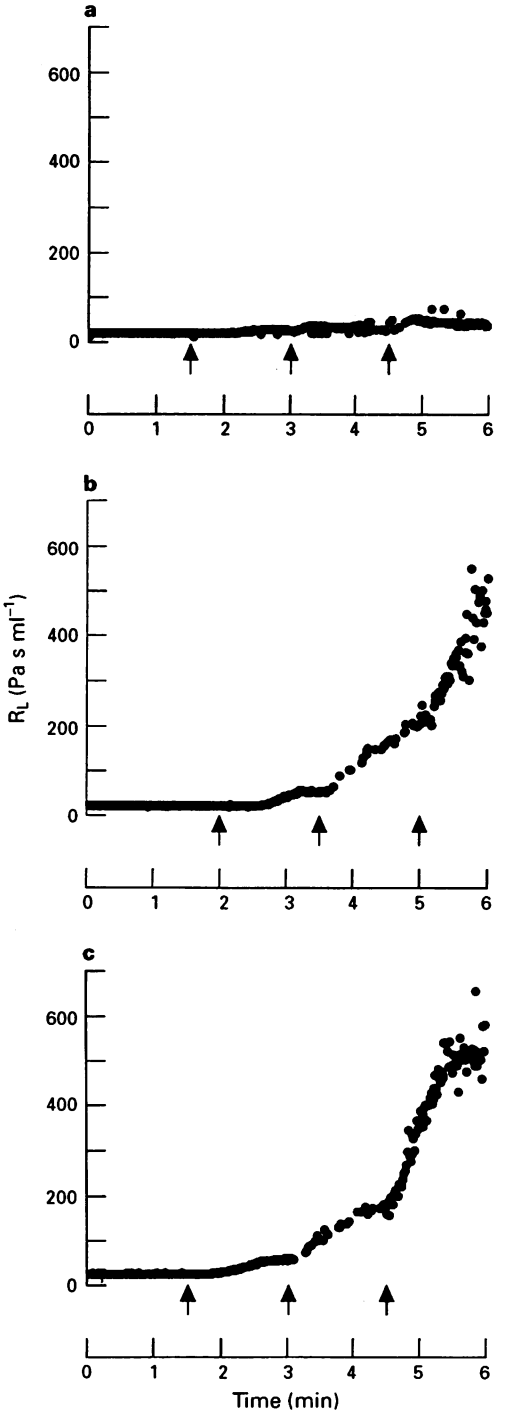

Figure 2 Enhancement by (±)-isoprenaline of responses to histamine. Breath-by-breath values of R_L recorded in response to successively increased rates of i.v. infusion of histamine (12, 24 and 48 μg min^{-1} kg^{-1} at arrows) in one guinea-pig 20 min before (a), and 20 min (b) and 90 min (c) after a 30-min infusion of (±)-isoprenaline (0.4 μmol h^{-1} kg^{-1}).

fall. For example, the mean fall produced by isoprenaline in 10 animals was 17.43 ± 1.71 mmHg. Falls in blood pressure were maximal during the first few min of infusion but partly recovered as the infusion continued. With each drug, blood pressure returned to pre-infusion levels within 20 min of stopping the infusion. Infusion of (+)-isoprenaline had no effect.

The i.v. infusions of histamine used to determine airway reactivity consistently lowered arterial blood pressure. Mean falls in blood pressure were not related to the histamine infusion rate. They were, however, dependent on the initial blood pressure. In 12 animals, the mean fall producd by the lowest histamine infusion rate before infusion of the β-agonists was 12.4 ± 3.17 mmHg.

Effect of (±)-isoprenaline infusion on bronchial reactivity to histamine

Figure 2 shows the effect on bronchial reactivity to histamine of infusing (±)-isoprenaline at 0.4 μmol h^{-1} kg^{-1} for 30 min in one guinea-pig. Breath-by-breath changes in R_L produced by cumulative increases in histamine dose rate recorded 20 and 90 min after stopping the infusion of (±)-isoprenaline were greatly enhanced, particularly at the higher dose rates of histamine (Figures 2b and 2c).

Figure 3a shows the significant enhancement ($P<0.05$) of the mean % increase in R_L produced by histamine measured in 4 animals 20 and 90 min after stopping the 30-min infusion of (±)-isoprenaline. This confirmed a similar observation made in a preliminary study in 6 animals (Galland & Blackman, 1988). In both series of experiments, the enhancement at 90 min did not differ significantly ($P>0.05$) from that observed at 20 min. Enhancement was observed whether measurements were of R_L or C_{dyn}^{-1}.

The infusion of (±)-isoprenaline did not enhance effects of histamine on blood pressure and heart rate. Infusions of saline in 6 control animals had no effect on bronchoconstrictor responses to histamine (Figure 3b).

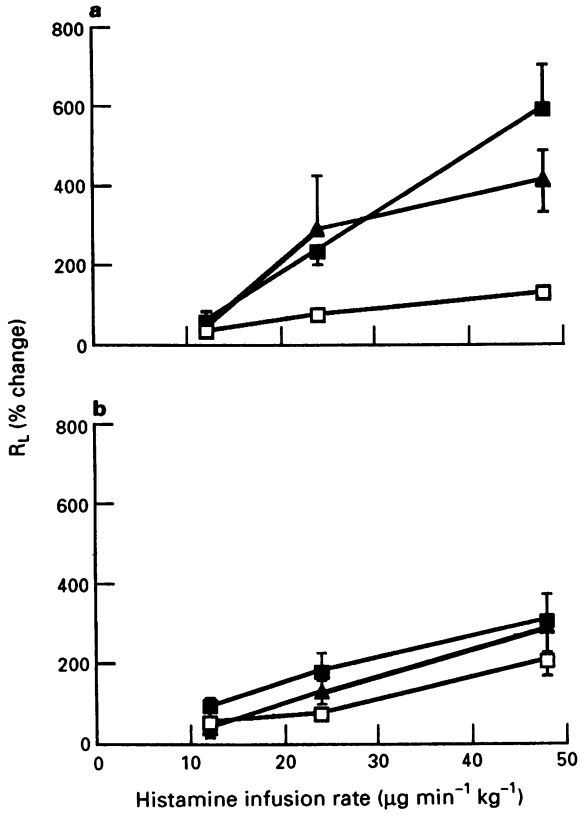

Figure 3 Changes in R_L in response to successively increased rates of i.v. infusion of histamine 20 min before (□), and 20 min (■) and 90 min (▲) after an infusion of either 0.4 μmol h^{-1} kg^{-1} (±)-isoprenaline in 4 animals (a) or saline in 6 animals (b); s.e.mean shown by vertical bars. Resting values of R_L (Pa s ml^{-1}) for the 3 periods of measurement in (a) were: 23.5 ± 2.7, 21.0 ± 1.9 and 21.5 ± 2.2 respectively, and for (b): 24.0 ± 2.0, 20.7 ± 0.7 and 22.5 ± 1.3 respectively.

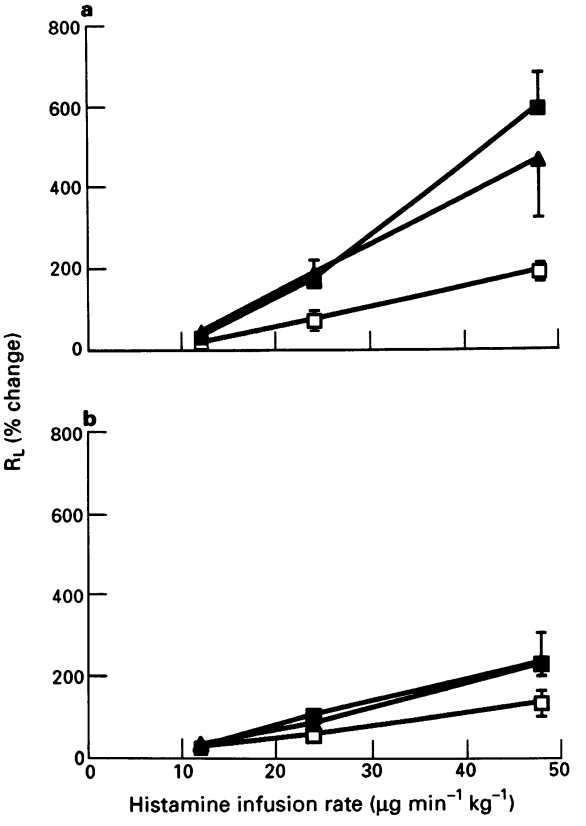

Figure 4 Comparison of the effects of the two optical isomers of isoprenaline. (a) Changes in R_L in response to successively increased rates of i.v. infusion of histamine 20 min before (□), and 20 min (■) and 90 min (▲) after an infusion of (−)-isoprenaline (0.2 μmol h^{-1} kg^{-1}) in 5 animals; s.e.mean shown by vertical bars. Resting values of R_L (Pa s ml^{-1}) for the 3 periods of measurement were: 18.5 ± 0.6, 18.2 ± 0.4 and 19.7 ± 0.9 respectively. (b) The changes in R_L in response to successively increased rates of i.v. infusion of histamine 20 min before (□), and 20 min (■) and 90 min (▲) after infusion of (+)-isoprenaline (0.28 μmol h^{-1} kg^{-1}) in 4 animals; s.e.mean shown by vertical bars. Resting values of R_L (Pa s ml^{-1}) for the 3 periods of measurement were: 20.8 ± 2.1, 20.3 ± 2.0 and 20.8 ± 1.7 respectively.

Effect of the (−)- and (+)-isomers of isoprenaline

Infusion of (−)-isoprenaline for 30 min at a dose rate of 0.2 μmol h^{-1} kg^{-1} in 5 animals significantly enhanced ($P<0.01$) airway responses to histamine (Figure 4a). Infusions at 0.4 and 0.8 μmol h^{-1} kg^{-1}, each in 4 animals, also increased reactivity (no figure shown), but not to a greater extent than at the low dose rate. The enhancement of reactivity at the dose rate of 0.2 μmol h^{-1} kg^{-1} appeared therefore to be maximal.

In contrast, infusion of (+)-isoprenaline for 30 min at a dose rate of 0.28 μmol h^{-1} kg^{-1} in 4 animals failed ($P>0.05$) to enhance responses to histamine (Figure 4b). Infusion at 0.4 μmol h^{-1} kg^{-1} in one animal and at 0.8 μmol h^{-1} kg^{-1} in two animals failed also to enhance responses to histamine.

Effects of adrenaline and salbutamol

Figure 5a shows the effect of adrenaline infused at 0.3 μmol h^{-1} kg^{-1} for 30 min on bronchial reactivity to histamine in one animal. As with (±)-isoprenaline, breath-by-breath changes in R_L in response to histamine were markedly enhanced (Figures 5b and 5c).

Figure 6a shows the mean % increase in R_L produced by histamine after infusion of adrenaline in 6 animals. Control infusions in 4 animals of 0.9% NaCl containing Na metabisulphite at the concentration present in the adrenaline used for infusion had no effect on reactivity to histamine.

Figure 6b shows that infusion of salbutamol at 0.4 μmol h^{-1} kg^{-1} for 30 min in 4 animals significantly enhanced ($P<0.05$) reactivity to histamine 90 min after stopping the infusion, but not 20 min after. At this time, the absolute value of R_L had not recovered to its pretreatment value (Figure 1a), indicating that the bronchodilator effect of salbutamol was still present 20 min after stopping the infusion.

Effect of vagotomy on isoprenaline-induced increase in reactivity to histamine

Acute bilateral vagotomy alone had no significant effect on baseline levels of R_L or C_{dyn}^{-1} or on responses to histamine measured 50 min after transection of the vagus nerves in 6 animals (Figure 7). This figure shows that after vagotomy, infusion of isoprenaline could still induce enhanced reactivity to histamine, but only at the highest dose rate ($P<0.01$); no enhancement occurred at the lower dose rates of histamine. However, comparison of the dose-response curves in Figure 7 and Figure 3a indicates that vagotomy had no significant effect on the degree of enhancement of reactivitiy to histamine ($P>0.05$).

Reactivity of bronchial smooth muscle

Isoprenaline (1 μmol l^{-1}) initially decreased resting tension in isolated preparations of first-branch bronchi. Tension recov-

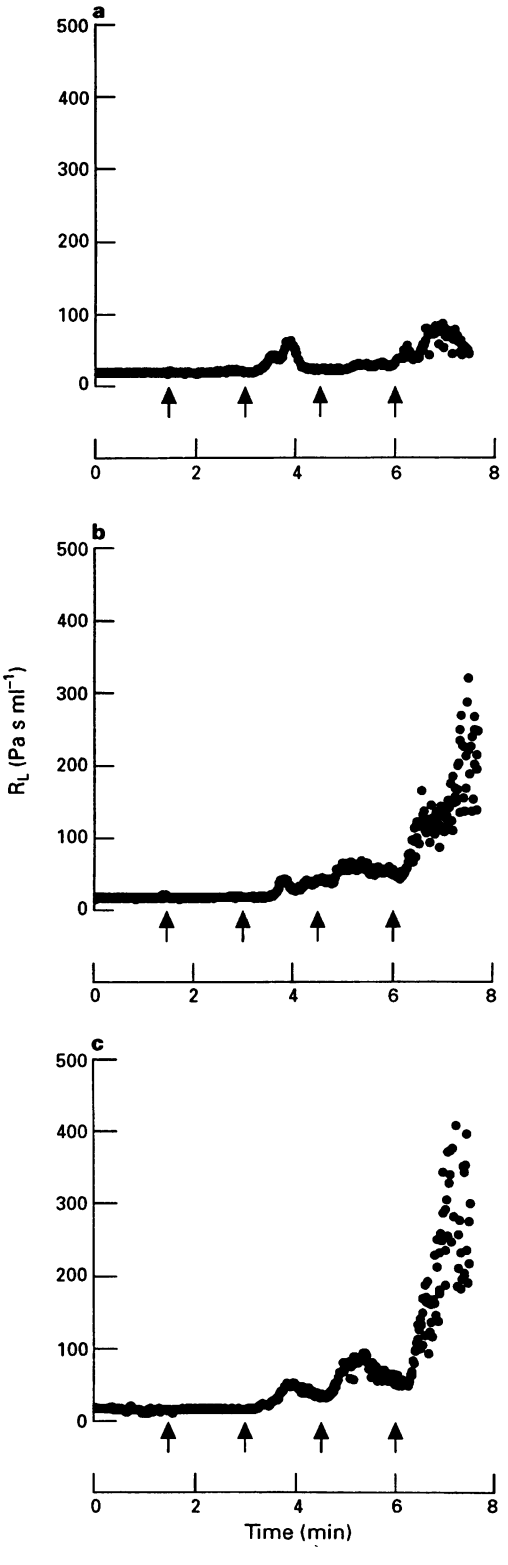

Figure 5 Enhancement by adrenaline of responses to histamine. Breath-by-breath values of R_L recorded in response to successively increased rates of i.v. infusion of histamine (12, 24, 48 and 96 μg min^{-1} kg^{-1} at arrows) in one guinea-pig 20 min before (a), and 20 min (b) and 90 min (c) after a 30-min infusion of adrenaline (0.3 μmol h^{-1} kg^{-1}).

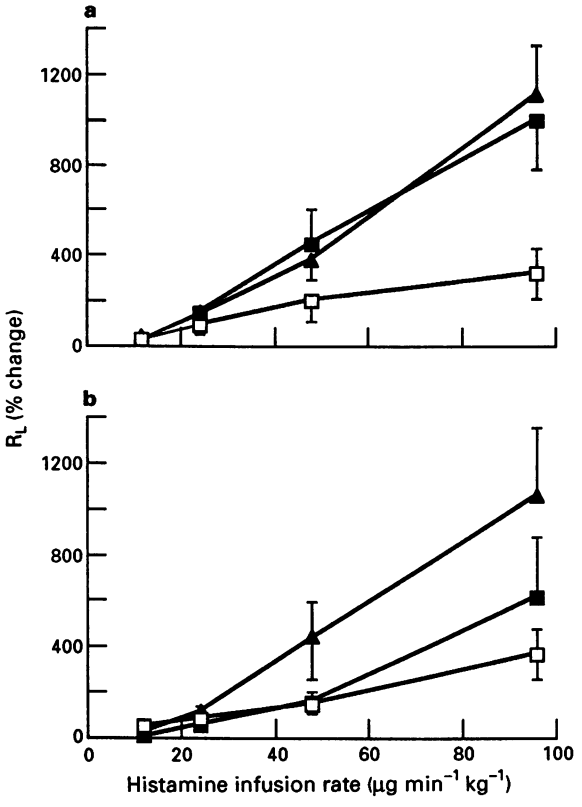

Figure 6 Changes in R_L in response to successively increased rates of i.v. infusion of histamine 20 min before (□), and 20 min (■) and 90 min (▲) after 30-min infusions of 0.3 μmol h^{-1} kg^{-1} adrenaline in 6 animals (a) and 0.4 μmol h^{-1} kg^{-1} salbutamol in 4 animals (b); s.e.mean shown by vertical bars. Resting values of R_L (Pa s ml^{-1}) for the 3 periods of measurement in (a) were: 21.4 ± 2.4, 19.6 ± 1.9 and 19.6 ± 1.3 respectively, and for (b); 23.5 ± 0.7, 17.3 ± 1.8 and 19.8 ± 2.7 respectively.

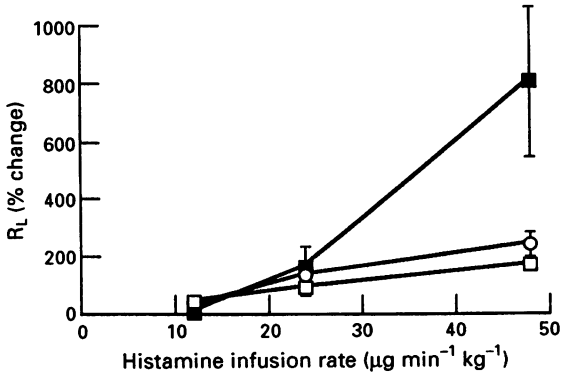

Figure 7 The change in R_L in response to successively increased rates of i.v. infusion of histamine 20 min before (□) and 50 min following bilateral vagotomy (○), and then 20 min after a 30-min infusion of 0.4 μmol h^{-1} kg^{-1} (±)-isoprenaline (■) in 6 animals; s.e.mean shown by vertical bars. Resting values of R_L (Pa s ml^{-1}) for the 3 periods of measurement were: 28.8 ± 5.3, 25.0 ± 3.2 and 22.8 ± 2.4 respectively.

ered to near normal during the 30 min exposure. Sensitivity to histamine did not change significantly after 30 min exposure to isoprenaline (Figure 8): values of EC_{50} before and after treatment were respectively 0.8 ± 0.1 and 1.5 ± 0.5 μmol l^{-1}. Maximal responses were not changed.

Discussion

Our observations confirm the principal findings reported by Morley and co-workers (Morley & Sanjar, 1987; Mazzoni *et al.*, 1987; Sanjar *et al.*, 1990; Morley *et al.*, 1991) that i.v. infusions of isoprenaline or salbutamol enhance airway reac-

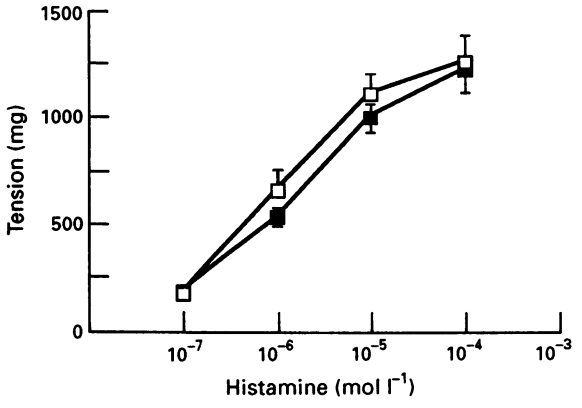

Figure 8 Responses of 8 isolated first-branch bronchi to cumulatively increased concentrations of histamine before (□) and after (■) exposure to (±)-isoprenaline (1 μmol l^{-1}); s.e.mean shown by vertical bars.

tivity in anaesthetized guinea-pigs. However, some of our results differ and require some discussion.

First, we found that adrenaline enhanced reactivity to histamine about as effectively as a similar concentration of isoprenaline. This is at variance with the observations of Sanjar *et al.* (1990) who observed no enhancement. The difference can perhaps be explained by inspection of our Figure 6a. In the absence of adrenaline, the two lower dose rates of histamine produced increases in R_L ranging from less than 150% to 250%. The bolus test doses of bombesin or histamine used by Sanjar *et al.* (1990) caused increases in R_L of not more than about 140%, which in our experiments would not have been enough to reveal significant hyperreactivity. Our observations with adrenaline (and also salbutamol) are consistent with reports that increased sensitivity to allergen in guinea-pigs occurs following pretreatment with these agonists (Izard *et al.*, 1971; Chapman *et al.*, 1990).

Secondly, our experience with vagotomy was different. Sanjar *et al.* (1990) reported that although bilateral vagotomy performed after infusion of isoprenaline was without effect on the development of hyperreactivity, vagotomy carried out before infusion of isoprenaline prevented enhancement of reactivity from which they concluded that development of hyperreactivity depended on intact innervation of the airways by the vagus. We also found that bilateral vagotomy carried out before infusion of isoprenaline prevented enhancement of reactivity, but only at the lower dose rates of histamine. At the highest dose rate of histamine, vagotomy did not prevent enhancement by isoprenaline (compare Figures 3 and 7). It thus appeared that vagotomy had shifted the histamine dose-response curve somewhat to the right, consistent with the removal of vagal tone. Our observation is also consistent with the finding of Sanjar *et al.* (1990) that treatment with atropine before infusion of isoprenaline did not prevent the development of hyperreactivity. Our finding that bronchial smooth muscle responses to histamine *in vitro* were not enhanced by isoprenaline, supports the conclusion that isoprenaline does not affect the reactivity of the smooth muscle directly. This evidence together with the results observed following vagotomy suggests that isoprenaline enhances reactivity at a peripheral site both independent of vagal innervation and not directly involving the smooth muscle. In the absence of further evidence, it is not possible to propose a more specific site of action.

Thirdly, we have concluded that the (−)-isomer of isoprenaline induced hyperreactivity in our preparations, evidently by stereoselective activation of β-adrenoceptors since the (+)-isomer had little if any activity (Galland & Blackman, 1988). Sanjar *et al.* (1990) found on the other hand that the isomers were equally effective at increasing airway reactivity and concluded that the enhancement of response was not mediated by steroselective activation of β-adrenoceptors. Because in our preparations (+)-isoprenaline did not enhance reactivity, we checked the identity of our sample. The specific optical rotation and infra-red spectrum were consistent with its being the (+)-isomer. Its lack of hypotensive effect was also consistent. We can offer no explanation for the different outcome in the two studies. Since ours is a negative finding, we hesitate without further study to contradict the conclusion that isoprenaline and related compounds produce hyperreactivity by a mechanism not involving stereoselective activation of β-adrenoceptors, particularly since the non-bronchodilator isomer (+)-salbutamol has been shown to enhance airway reactivity (Morley *et al.*, 1991).

We also found that (±)-salbutamol increased airway reactivity to histamine, and like Morley *et al.* (1991) noted that the longer bronchodilator action of this drug prevented observation of an early increase in reactivity. Perhaps because we infused salbutamol for a shorter time, we were able to observe hyperreactivity at 90 min.

The differences we have discussed possibly reflect differences in the methods used in the two studies to determine airway reactivity. In particular, we plotted cumulative dose-response curves to increasing i.v. infusion rates of a fixed concentration of histamine whereas Sanjar *et al.* (1990) measured peak responses to single i.v. bolus doses of bombesin or histamine. Although the normal dose-response relationship to histamine inferable from their published record (Sanjar *et al.*, 1990, Figure 3) is approximately the same as that found in our experiments (our Figure 2a), the difference in the method of determining reactivity was substantial.

A feature of the hyperreactivity produced by i.v. infusion of a β-agonist is that it is an acute response; it is observed as soon as the bronchodilator effect has waned. The effect is surprisingly long-lasting. In our experiments, hyperreactivity was unabated 90 min after stopping infusion of the β-agonist, and in one set of experiments by Morley and colleagues (Sanjar *et al.*, 1990), hyperreactivity was observed 4 h after stopping the infusion. Other evidence indicates that it may last even longer (Morley *et al.*, 1991).

Whatever the mechanism of this acute effect of β-agonists given i.v., an important question is whether β-agonists can be shown to increase airway reactivity acutely in man. Indirect evidence of an acute increase in reactivity following administration of β-agonists by inhalation has been described, but only in asthmatic subjects (Keighley, 1966; Eisenstadt & Nicholas, 1969; van Metre, 1969; Reisman, 1969; Paterson *et al.*, 1971). We are not aware of studies designed to test directly the possibility that the effect occurs in man with normal respiratory function following either i.v. administration or inhalation. Nor are we aware of experiments designed to see whether inhalation of β-agonists increases bronchial reactivity in guinea-pigs or other animals. Study of the effect of β-agonists in normal human subjects would parallel more strictly the observations in animals and would indicate that the effect could occur independently of the disease process.

Other studies indicate that enhanced airway reactivity can result from repeated exposure to conventional β-agonist bronchodilator drugs in asthmatic patients being treated with these agents prophylactically (Peel & Gibson, 1980; Kraan *et al.*, 1985; Kerribijn *et al.*, 1987; Vathenen *et al.*, 1988; van Schayck *et al.*, 1990; Sears *et al.*, 1990). Regular daily use of a β-agonist has been shown to worsen the disease and make control of symptoms difficult (Sears *et al.*, 1990) and to be associated with an increase in asthma mortality (Crane *et al.*, 1989; Spitzer *et al.*, 1992). Morley *et al.* (1990), reviewing reports of 'anomalous'or 'paradoxical bronchospasm' in asthma patients treated with β-agonists, considered five possible explanations, namely, reactive myogenic tone, metabolic products, tachyphylaxis to the β-agonist, increased inflammatory burden and induction of airway hyperreactivity and decided that only the last seemed able to explain the observations.

If β-agonists acutely enhance airway reactivity in man, why

has the effect not been more readily demonstrable in asthmatic patients being treated with these agents? A possible answer is that therapeutic doses in man do not achieve concentrations at the site of action equivalent to those reached in the guinea-pig. However, the evidence suggests otherwise. For example, in severe asthma, a typical bronchodilator infusion rate of salbutamol is 10 $\mu g\,min^{-1}$ i.v., which is equivalent to 0.035 $\mu mol\,h^{-1}\,kg^{-1}$, a little less than one tenth the rate used in our guinea-pigs. Allowing for the effect of scale (Gibaldi & Perrier, 1982), clearance of salbutamol in relation to body wt should be significantly greater in the guinea-pig than in man, so that the steady-state concentrations reached in the two species should be of the same order. Moreover, the concentrations needed to induce hyperreactivity may be much lower. Isoprenaline infused at 1 $\mu g\,h^{-1}\,kg^{-1}$ (approximately 0.004 $\mu mol\,h^{-1}\,kg^{-1}$) can significantly enhance reactivity in the guinea-pig (Sanjar *et al.*, 1990). It is reasonable, therefore, to suppose that the bronchodilator doses used in man are at least equivalent to those shown to induce hyperreactivity in the guinea-pig.

At least part of the answer as to why enhancement of reactivity by β-agonists has not been more readily demonstrable is that few studies with β-agonists have been designed to detect it, and when they have (as noted above), evidence of only a limited enhancement has been obtained. Another part of the answer, is that any acute increase in airway hyperreactivity will be detected only if it outlasts the bronchodilator or other beneficial effects of β-agonist treatment. In the guinea-pig, the induced hyperreactivity long outlasts the initial bronchodilator effect, but in man, the pharmacokinetic behaviour of the β-agonists appears to ensure that the bronchodilator effect is sustained longer. Also, and perhaps more importantly, since the airways in the more severely affected asthmatic patient already exhibit marked hyperresponsiveness, any additional effect of a β-agonist may represent only a small relative increase. Nevertheless, a small increase may be critical for the survival of a patient suffering a life-threatening asthmatic attack. Enhancement of reactivity is likely to be more obvious in the mild to moderate asthmatic patient, and indeed, it is in this group of patients that evidence of enhanced reactivity has been detected. Experiments directly showing that β-agonists can or cannot acutely enhance airway reactivity in normal and asthmatic human subjects are needed to settle such speculative argument.

We thank Murray Craig for technical assistance, Dr Robert Purves for writing the programmes for on-line computation and Dr David Schmierer of the Otago University School of Pharmacy for performing the optical and and infra-red analyses. The work, which was part of a thesis submitted for the award of a Ph.D. (Galland, 1989), was supported by a grant from the Dean's Fund, Otago Medical School, and in part by the Medical Research Council of New Zealand. The experiment protocol was approved by the Otago Medical School Committee on Ethics in the Care and Use of Laboratory Animals.

References

AMDUR, M.O. & MEAD, J.M. (1958). Mechanics of respiration in unanaesthetized guinea-pigs. *Am. J. Physiol.*, **192**, 364–368.

BOUHUYS, A., DOUGLAS, J.S. & LEWIS, A.J. (1972). Hypersensitivity to adrenoceptor agents in the guinea-pig *in vitro* and *in vivo*. *Br. J. Pharmacol.*, **46**, 520–521P.

CHAPMAN, I., MAZZONI, L. & MORLEY, J. (1990). An anomalous effect of salbutamol in sensitised guinea-pigs. *Br. J. Pharmacol.*, **99**, 66P.

CONOLLY, M.E., DAVIES, D.S., DOLLERY, C.T. & GEORGE, C.F. (1971). Resistance to β-adrenoceptor stimulants (a possible explanation for the rise in asthma deaths). *Br. J. Pharmacol.*, **43**, 389–402.

CRANE, J., FLATT, A., JACKSON, R., BALL, M., PEARCE, N., BURGESS, C., KWONG, T. & BEASLEY, R. (1989). Prescribed fenoterol and death from asthma in New Zealand, 1981–1983: case-control study. *Lancet*, **i**, 917–922.

EISENSTADT, W.S. & NICHOLAS, S.S. (1969). The adverse effect of adrenergic aerosols in bronchial asthma. *Ann. Allergy*, **27**, 283–288.

GALLAND, B.C. (1989). Bronchial hyperreactivity in the guinea-pig. *Ph.D. thesis*. Dunedin: University of Otago Medical School.

GALLAND, B.C. & BLACKMAN, J.G. (1988). Enhancement of airways reactivity to histamine by (±)-isoprenaline and its isomers in guinea-pigs. *Proc. Univ. Otago Med. Sch.*, **66**, 25–26.

GALLAND, B.C. & BLACKMAN, J.G. (1989). Enhancement of airways reactivity to histamine in the guinea-pig by β-adrenoceptor agonists. *N.Z. Med. J.*, **102**, 226.

GALLAND, B.C., PURVES, R.D. & BLACKMAN, J.G. (1986). Bronchial hyper-reactivity in the immunologically sensitized guinea-pig. *Proc. Univ. Otago Med. Sch.*, **64**, 35–36.

GIBALDI, M. & PERRIER, D. (1982). *Pharmacokinetics*, 2nd ed. pp. 375–382. New York: Marcel Dekker.

HOOKER, C.S., CALKINS, P.J. & FLEISCH, J.H. (1977). On the measurement of vascular and respiratory smooth muscle responses *in vitro*. *Blood Vessels*, **14**, 1–11.

IZARD, S.R., HENSON, E.C., COLLINS, A.D. & BRUNSON, J.G. (1971). Increased sensitivity to anaphylactic shock in guinea-pigs induced by prolonged treatment with epinephrine prior to challenge. *J. Allergy*, **47**, 309–314.

KEIGHLEY, J.F. (1966). Iatrogenic asthma associated with adrenergic aerosols. *Ann. Intern. Med.*, **65**, 985–995.

KELLER, R.J. (1986). *Sigma Library of FTR Spectra*, Vol. 1, p. 673, spectrum A. St Louis: Sigma Chemical Co.

KERREBIJN, K.F., VAN ESSEN-ZANDVLIET, E.E.M. & NEIJENS, H.J. (1987). Effect of long-term treatment with inhaled corticosteroids and beta-agonists on the bronchial responsiveness in children with asthma. *J. Allergy Clin. Immunol.*, **79**, 653–659.

KRAAN, J., KOETER, G.H., VAN DER MARK, TH. W., SLUITER, H.J. & DE VRIES, K. (1985). Changes in bronchial hyperreactivity induced by 4 weeks of treatment with antiasthmatic drugs in patients with allergic asthma: a comparison between budenoside and terbutaline. *J. Allergy Clin. Immunol.*, **76**, 628–636.

MAZZONI, L., MORLEY, J., SANJAR, S. & SCHAEUBLIN, E. (1987). An anomalous interaction between propranolol and isoprenaline. *Br. J. Pharmacol.*, **91**, 326P.

MORLEY, J. & SANJAR, S. (1987). Isoprenaline induces increased airway reactivity in the guinea-pig. *J. Physiol.*, **390**, 180P.

MORLEY, J., SANJAR, S. & NEWTH, C. (1990). Viewpoint: untoward effects of beta-adrenoceptor agonists in asthma. *Eur. Resp. J.*, **3**, 228–233.

MORLEY, J., CHAPMAN, I.D., FOSTER, A., HOSHIKO, K. & MAZZONI, L. (1991). Effects of (+) and racemic salbutamol on airway responses in the guinea-pig. *Br. J. Pharmacol.*, **104**, 295P.

PATERSON, J.W., EVANS, R.J.C. & PRIME, F.J. (1971). Selectivity of bronchodilator action of salbutamol in asthmatic patients. *Br. J. Dis. Chest*, **65**, 21–38.

PEEL, E.T. & GIBSON, G.J. (1980). Effects of long term inhaled salbutamol therapy on the provocation of asthma by histamine. *Am. Rev. Respir. Dis.*, **121**, 317–321.

REISMAN, R.E. (1969). Asthma induced by adrenergic aerosols. *J. Allergy*, **46**, 162–177.

SANJAR, S., KRISTERSSON, A., MAZZONI, L., MORLEY, J. & SCHAEUBLIN, E. (1990). Increased airway reactivity in the guinea-pig follows exposure to intravenous isoprenaline. *J. Physiol.*, **425**, 43–54.

SEARS, M.R., TAYLOR, D.R., PRINT, C.G., LAKE, D.C., LI, Q., FLANNERY, E.M., YATES, D.M., LUCAS, M.K. & HERBISON, G.P. (1990). Regular inhaled beta-agonist treatment in asthma. *Lancet*, **336**, 1391–1396.

SPITZER, W.O., SUISSA, S., ERNST, P., HORWITZ, R.I., HABBICK, B., COCKCROFT, D., BOIVIN, J.-F., MCNUTT, M., BUIST, A.S. & REBUCK, A.S. (1992). The use of β-agonists and the risk of death and near death from asthma. *New Engl. J. Med.*, **326**, 501–506.

VAN METRE, T.E. (1969). Adverse effects of inhalation of excessive amounts of nebulized isoproterenol in status asthmaticus. *J. Allergy*, **43**, 101–113.

VAN SCHAYCK, C.P., GRAAFSMA, S.J., VISCH, M.B., DOMPELING, E., VAN WEEL, C. & VAN HERWAARDEN, C.L.A. (1990). Increased bronchial hyperresponsiveness after inhaling salbutamol during 1 year is not caused by subsensitization to salbutamol. *J. Allergy Clin. Immunol.*, **86**, 793–800.

VATHENEN, A.S., KNOX, A.J., HIGGINS, B.G., BRITTON, J.R. & TATTERSFIELD, A.E. (1988). Rebound increase in bronchial responsiveness after treatment with inhaled terbutaline. *Lancet*, **i**, 554–558.

(Received August 14, 1992
Revised November 24, 1992
Accepted December 3, 1992)