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The effects of formoterol on plasma exudation produced by a localized acute inflammatory response to bradykinin in the tracheal mucosa of rats *in vivo*

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1 The effects of formoterol, a β_2-adrenoceptor agonist, on plasma protein exudation and microvascular permeability induced by topical, i.e. applied onto the tracheal mucosal surface, bradykinin (10 nmol; 20 μM, 5 min, 0.1 ml min^{-1}) were studied in a perfused segment of trachea prepared *in situ* in anaesthetized rats.

2 Bradykinin increased the amount of plasma (fluorimetric assay for protein) in the perfusate (response; 10.98 ± 0.357 μl, $n=69$; total increase in plasma over basal during 45 min after start of bradykinin application) and 2 responses at a 90 min interval were reproducible. Carbon labelling was seen in tracheal sections from animals that received i.v. colloidal carbon, indicating that bradykinin caused tracheal microvessels to leak (increase in microvascular permeability).

3 Five minutes after topical formoterol, 5 or 30 nmol (10 or 60 μM perfused for 5 min), the bradykinin response was significantly reduced. The effects of formoterol were not dose-related, i.e. were maximal at 5 nmol. The bradykinin response was at control levels 30 min after 5 nmol formoterol. After 30 nmol formoterol, the response was still reduced 120 min later. The bradykinin response was significantly reduced 60 min after systemic formoterol (i.p., 0.029 to 870 nmol kg^{-1}) and, for 290 nmol kg^{-1} i.p. formoterol, this reduction was shown to last at least 150 min.

4 The bradykinin response was not prevented by supramaximal doses of topical (30 nmol) or i.p. (870 nmol kg^{-1}) formoterol and carbon-labelled microvessels were seen in tracheal sections from all animals that received formoterol, although these were less in number and less densely labelled than in the absence of formoterol. There was a correlation between the plasma exudation response (μl) and the number of carbon-labelled vessels (Spearman's correlation coefficient 0.415, $P<0.001$).

5 In animals pretreated with propranolol (3 μmol kg^{-1}, i.v.), 29 nmol kg^{-1} formoterol, i.p., did not reduce the bradykinin response. However, propranolol itself markedly potentiated the bradykinin response which confounded the interpretation of its effects on formoterol.

6 The study has shown, in a preparation of rat trachea *in situ*, that supramaximal doses of the β_2-adrenoceptor agonist, formoterol (a) produced a sustained, but incomplete, inhibition of plasma exudation (induced by topical bradykinin), and (b) did not prevent bradykinin-induced leaky microvessels. The data support the view that, at least in rodent airways, β_2-adrenoceptor agonists attenuate, but do not abolish, the microvascular permeability effects of bradykinin, a putative asthma mediator.

Keywords: Formoterol; plasma exudation; acute inflammatory responses; bradykinin; microvascular permeability; rat trachea *in vivo*

Introduction

Inflammatory mediators in asthmatic airways cause an increase in the permeability of microvessels in the tracheobronchial region and the exudation of plasma. Proteins from the plasma exudate can be detected in the airway tissue and the airway lumen (see Discussion) and it has been suggested that plasma exudate may contribute to the pathophysiology of asthma (Persson, 1986; 1988).

β_2-Selective adrenoceptor agonists have been shown to suppress microvascular leakage and/or plasma exudation responses associated with an acute inflammatory challenge in the tracheo-bronchial airways in a number of animal models (Persson & Erjefält, 1979; O'Donnell & Wholohan, 1984; Tokuyama *et al.*, 1991; Erjefält & Persson, 1991; 1992; Persson, 1993). The aim of the present study was to examine the effects of formoterol, given either topically (onto the mucosal surface) or systemically (i.p.), on the acute plasma protein exudation and microvascular leakage responses to bradykinin, which was applied topically onto the tracheal mucosal surface. Formoterol is a potent, highly β_2-selective adrenoceptor agonist, which was first synthesized and studied in animals, as BD40A, in the 1970s (Ida, 1976a, b). It has recently been introduced for the treatment of reversible airway obstruction in diseases such as asthma. In clinical practice, the bronchodilator effect of formoterol is very rapid in onset and lasts in excess of 12 h when given by the inhaled route (Anderson, 1991; 1993).

The rat model used in the present study involved luminal perfusion of a segment of trachea *in situ* in anaesthetized animals and regular collection of the perfusate over a period of about 3 h. In contrast to most previous studies on airway plasma exudation or microvascular leakage, this allowed continuous measurement of the amount of plasma in the tracheal lumen and a study of the response to bradykinin before, and at various times after, the administration of formoterol.

Preliminary communications on these data were presented to the British Pharmacological Society (O'Donnell & Anderson, 1991) and to the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (O'Donnell, 1992).

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Methods

Animals

Male, pathogen-free, Wistar rats, weighing 300–350 g were anaesthetized with fentanyl-fluanisone and midazolam (3.3 ml kg^{-1}, i.p. of a 1:1 mixture of a 50% dilution of fentanyl-fluanisone and a 50% dilution of midazolam in water). Anaesthesia was maintained with i.m. injections of fentanyl-fluanisone (0.15 ml kg^{-1}, every 20 min) as described by Flecknell (1987). The body temperature of the rats was maintained constant at 36–37°C throughout the experiment.

Experimental method

A cannula was inserted into the jugular vein, for injection of carbon tracer or drugs, and a segment of trachea was carefully prepared for perfusion, as described by Miller-Larsson & Brattsan (1991) and O'Donnell *et al.* (1991). A cannula (for spontaneous respiration) was placed in a small incision between the 9th and 10th cartilage rings from the larynx and directed caudally. Another cannula (to allow the inflow of the perfusion fluid) was firmly secured in the same incision and directed cranially. A cannula for collection of the outflow perfusion fluid was inserted into a small incision in the larynx and directed caudally. Care was taken to minimize bleeding, to avoid damage to the trachea and not to disturb the circulation or the innervation to the region. The length of the perfused tracheal region was 7 cartilages (approx. 1 cm).

Experimental protocol

The prepared tracheal segment was perfused with normal saline at 0.1 ml min^{-1} and room temperature (22°C). The saline contained formoterol, when indicated in the text, and captopril (10 μM) and thiorphan (10 μM) to inhibit the breakdown of bradykinin by peptidase enzymes.

The first 15 min of perfusate was discarded. During this time period a blood sample (0.3 ml) was taken to provide plasma for the reference curve for the fluorimetric assay of experimental samples (see below). Five min samples of perfusate (0.5 ml) were then collected into pre-weighed microfuge tubes for at least 30 min (to provide basal readings). This was followed by a 5 min perfusion with bradykinin (see Results for details) and the collection of further samples for 45 min. After discarding the next 15 min of perfusate, samples were collected before (for 30 min) and after (for 45 min) a second perfusion with bradykinin.

Some animals in each group were injected i.v. with 0.3 ml of a solution of colloidal carbon (1:1 dilution in normal saline). This was given 1 min after starting either the first or the second bradykinin perfusion, depending on the experiment. In some experiments, formoterol was applied topically to the tracheal mucosal surface by including it in the perfusion fluid at times indicated in the Results. In other experiments formoterol was administered i.p. Some animals received propranolol (3 μmol kg^{-1}, i.v.) 5 min before formoterol treatment.

Animals were killed at the end of the experiment with an overdose of sodium pentobarbitone and, if carbon had been given, the middle segment of the perfused tracheal region was prepared for histology. Carbon-labelled microvessels in the submucosal region of the trachea were examined as described previously (O'Donnell *et al.*, 1987; O'Donnell & Barnett, 1987; 1990).

Treatment of experimental samples

The blood sample was centrifuged (11,600 *g*, 10 min) and the plasma removed. All experimental samples of perfusate were also centrifuged and inspected to check for the absence of blood. If blood was present the experiment was terminated. The plasma was diluted in normal saline (dilution range 1 in 20,000 to 1 in 2000, equating to 0.05 to 0.5 μl plasma ml^{-1}) and the fluorescence (F, arbitrary values) of each dilution measured for protein native fluorescence (excitation wavelength 295 nm, emission wavelength 340 nm) as described by Miller-Larsson & Brattsand (1991).

The regression of *F* against plasma concentration (μl ml^{-1}) was calculated by Linear Least Squares Regression analysis (Snedecor & Cochran, 1967). Fluorescence readings were then obtained for all the experimental samples (diluted 1 in 10) and converted to μl ml^{-1} plasma by interpolation from the plasma regression line for that animal. The volume (μl) of plasma in each 5 min sample was obtained after adjustment for the exact volume (weight) of the sample. It was demonstrated that none of the drugs present in the perfusion solution, *viz.* bradykinin, captopril, thiorphan or formoterol, affected the location of the regression line for plasma diluted in normal saline.

Calculation of the responses to bradykinin

Where reference is made to a bradykinin response, this represents the total increase (over the basal value) in plasma in the perfusate (μl) during a period of 45 min after the start of the bradykinin perfusion (i.e. in 9 consecutive samples). The basal level of plasma in the perfusate was calculated from the three samples collected immediately before the beginning of each bradykinin perfusion (expressed in the Results in μl min^{-1}). If the basal value in an experiment exceeded 0.15 μl min^{-1}, the results for that experiment were not used.

Statistical analyses

Mean values for bradykinin responses are shown with their standard errors (s.e.mean) since these data were normally distributed. The significance of differences between mean values have been assessed by Student's *t* test or paired *t* test, as indicated in the text. Other statistical procedures are indicated in the text.

Drugs and solutions

The drugs and chemicals used were: bradykinin acetate (Sigma), budesonide (Astra-Draco), captopril (Squibb), colloidal carbon (Pelikan indian ink, filtered), fentanyl-fluanisone (0.2 mg ml^{-1} and 10 mg ml^{-1} respectively, Hypnorm vet, Janssen-Cilag), (RR,SS) formoterol (2-hydroxy-5-[(1**RS**)-hydroxy-2-[[(1**RS**-2-(*p*-methoxyphenyl)-1-methylethyl]amino] ethyl] formanilide fumarate dihydrate, aformoterol, Foradil, Ciba-Geigy, Switzerland), midazolam (5 mg ml^{-1}, Hypnovel, Roche); propranolol (ICI, U.K.), sodium pentobarbitone (Nembutal), thiorphan (Sigma).

Bradykinin (1 mM in distilled water) was stored in aliquots at −4°C. Thiorphan (10 mM in 0.2% alcohol) was stored at −4°C. On the day of the experiment, an aliquot of bradykinin was diluted as required, formoterol (1 mM in distilled water) and captopril (10 mM in normal saline) were prepared, and captopril, thiorphan and formoterol were diluted as required. All dilutions were made in normal saline and these dilutions, as well as the stock solutions of captopril and formoterol, were discarded daily.

Results

Responses to bradykinin

Perfusion of 20 μM bradykinin over the tracheal mucosal surface for 5 min at 0.1 ml min^{-1}, representing application of 10 nmol, increased the amount of plasma in the perfusion fluid. The increase was seen 5 min after cessation of the bradykinin application and levels had returned to baseline 40 min later (Figure 1). In most experiments bradykinin was perfused twice at an interval of 90 min (these responses are referred to as BK1 and BK2); these responses were shown to be not significantly different (Figure 1). The mean responses (BK1) to

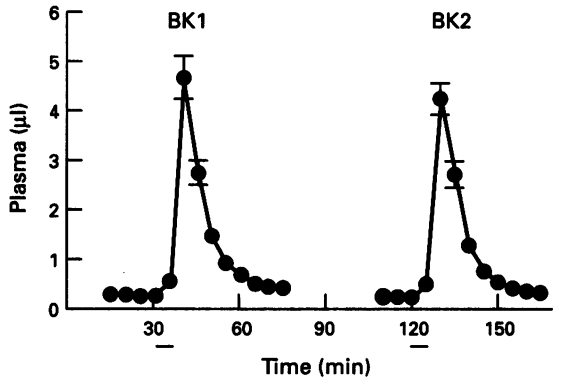

Figure 1 Plasma exudation responses to bradykinin in a tracheal segment in anaesthetized rats *in vivo*. The figure shows the 45 min time course of the increase in plasma that occurred after perfusion of bradykinin and the reproducibility of two bradykinin responses obtained 90 min apart in the same preparations. The tracheal segment was perfused with normal saline containing captopril and thiorphan (10 μM). Bradykinin (20 μM) was included in the perfusion fluid for 5 min at 30 and 120 min, as indicated by the horizontal bars (total dose applied 10 nmol). Points represent the mean amount of plasma (μl) collected in 5 min samples ($n=14$ animals); s.e.mean are shown only where these are greater than the size of the symbol. The first and second bradykinin responses were not significantly different (BK1 10.15 ± 1.068 μl plasma; BK2 9.11 ± 0.733, $n=14$, $P>0.05$ paired t test).

10 nmol bradykinin from 69 animals used in the study was 10.98 ± 0.367 μl (95% CL 10.25–11.71). The mean basal rate of plasma entry into the luminal fluid in the same animals was 0.075 ± 0.0045 μl min^{-1} (95% CL 0.062–0.084), i.e. 3.37 μl over 45 min.

Effects of topical formoterol

Five min after completion of the topical application of 5 nmol formoterol (perfusion of 10 μM at 0.1 ml min^{-1} for 5 min) the bradykinin response was significantly reduced (compared with the response in a group of control animals) but, at this dose, the responses 30, 95 and 120 min after formoterol were not significantly reduced (Figure 2a). In experiments in which a higher dose of formoterol was used (30 nmol; 60 μM at 0.1 ml min^{-1} for 5 min), the bradykinin response was significantly reduced at all the times studied (5, 30, 95 and 150 min after formoterol; Figure 2b). Comparison of the 5 min data, showed that the magnitude of the reduction by 30 nmol formoterol was no greater than that by 5 nmol formoterol ($P>0.05$; Student's t test). Comparison of the 120 min data, showed that the reduction of the bradykinin response after 30 nmol formoterol was significantly more than that after 5 nmol formoterol ($0.05>P>0.01$, Student's t test). These data suggest that increasing the dose of topical formoterol from 5 to 30 nmol did not increase the magnitude of its effect, i.e. the effect was maximal, but it did increase the duration of its effect.

Effects of systemic (i.p.) formoterol

The response to 10 nmol bradykinin was reduced 60 min after i.p. formoterol (0.029 nmol kg^{-1}, the lower dose tested). A number of higher doses of formoterol were tested in an attempt to abolish the bradykinin response but this was not achieved even with a dose of formoterol of 870 nmol kg^{-1} (Figure 3). The bradykinin response (BK2) in animals treated with a high dose of i.p. formoterol (870 nmol kg^{-1}, 60 min before BK2) plus 3 μM topical budesonide (perfused with the BK1, i.e. 90 min before BK2, as described by Brattsand *et al.*, 1991; 2.71 ± 0.728, $n=4$) was significantly less than the BK1 response in the same animals but was not significantly less than the BK2 response recorded in animals given only 870 nmol kg^{-1} i.p. formoterol (3.30 ± 0.075, $n=4$; data from Figure 3; $P>0.05$,

Table 1 Responses to 10 nmol bradykinin (20 μM perfused for 5 min) in animals pretreated with formoterol (i.p.), propranolol (i.v.) or propranolol and formoterol

Treatment before BK2	n	*Mean bradykinin response* (μl) *BK1* (control)	*BK2*
Formoterol	5	10.36 ± 0.77	$5.20\pm1.12^{\#\#}$
Propranolol	5	11.00 ± 1.06	$17.50\pm1.25^{*}$
Formoterol after propranolol	4	11.78 ± 2.27	11.65 ± 2.46

Values are means ± s.e.mean.
Formoterol (29 nmol kg^{-1}) was given i.p. 60 min before BK2.
Propranolol (3 μmol kg^{-1}) was given i.v. 5 min before formoterol. Normal saline was given i.p. 60 min before BK1 (control).
Significant decrease in the bradykinin response compared to control ($0.01>P>0.001$)
*Significant increase in the bradykinin response compared to control.

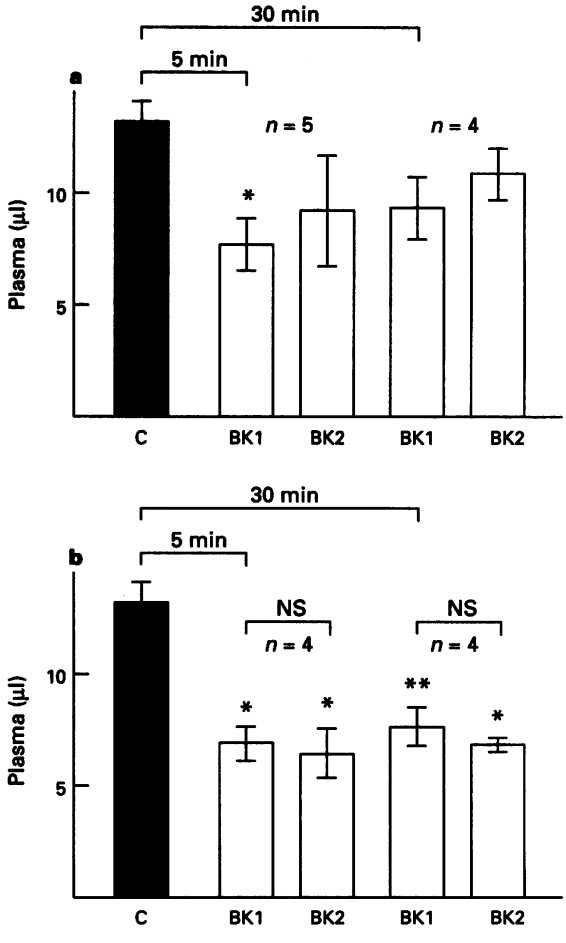

Figure 2 Concentration-dependence of the duration of formoterol inhibition of bradykinin-induced plasma exudation. In (a), 5 nmol formoterol was applied to the tracheal mucosa (10 μM was perfused for 5 min). In (b) 30 nmol formoterol was applied (60 μM was perfused for 5 min). Control responses (C) to bradykinin obtained in a group of animals given no formoterol are indicated by the solid columns ($n=4$). In test groups formoterol was applied to the mucosa either 5 min or 30 min before the first of two responses to superfused bradykinin (BK1) and the second response (BK2) was elicited 90 min later. Each pair of BK1 and BK2 responses was obtained in a separate group of animals ($n=4-5$). Data are expressed as mean ± s.e.mean. *$0.05>P>0.01$; **$0.01>P>0.001$; Dunnett's test for comparison of several treatments to a control. Data that are marked as not significantly different (NS) were compared by a paired t test.

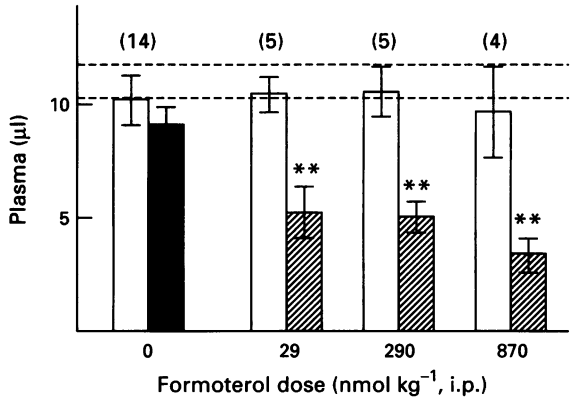

Figure 3 Effect of systemic formoterol on bradykinin responses obtained as in Figure 1. The open columns represent the control response to bradykinin in each group of animals (BK1). The solid column represents the second bradykinin response (BK2) in control animals (60 min after i.p. normal saline). In these animals the BK1 and BK2 responses were not significantly different (paired t test; $P>0.05$; data from Figure 1). The hatched columns represent the BK2 response 60 min after an i.p. injection of 29, 290 or 870 nmol kg^{-1} formoterol as indicated. In each of these groups of formoterol-treated animals the BK2 response was significantly less than the BK1 response (paired t test, $^{**}0.05>P>0.001$). Mean values are shown with s.e.mean. The numbers of animals in each treatment group are in parentheses. The 95% confidence limits of the bradykinin responses from all the control animals in the study ($n=69$, see text) are shown by the dotted lines, for comparison.

Student's t test), i.e. the response to bradykinin was not totally prevented by the actions of formoterol and budesonide.

In a group of animals given 290 nmol kg^{-1} i.p. formoterol, the bradykinin response was measured at 60 min and then at 150 min in the same animals. Both responses were less than that expected from the results in untreated animals (10.98 ± 0.367 µl, $n=69$) but the response at 150 min (7.720 ± 0.360, $n=4$) was significantly greater than that at 60 min (3.187 ± 0.360; $P<0.001$, paired t test). These data suggest that the effect of formoterol lasts at least 150 min but decreases with time after 1 h.

Effect of propranolol on formoterol

Experiments were carried out to examine whether the effects of formoterol were due to an action on β-adrenoceptors (Table 1). In animals pretreated with propranolol (3 µmol kg^{-1}, i.v.), formoterol (29 µmol kg^{-1}) did not attenuate the bradykinin response, compared with control bradykinin responses (no formoterol). However, propranolol itself markedly potentiated the bradykinin response, which confounded the interpretation of its effects on formoterol.

Carbon-labelled vessels in the trachea

Administration of i.v. carbon at the beginning of the bradykinin perfusion did not influence the magnitude of the bradykinin response.

In tracheal tissues from 8 control animals, i.e. that had not been treated with formoterol and/or propranolol, an average of 81 ± 4.9 carbon-labelled vessels per section was counted. Carbon-labelled vessels were still present in tissues taken from animals that had received formoterol but the numbers of vessels were significantly less than in controls (systemic formoterol, 57 ± 3.2, $n=30$, $0.01>P>0.001$, topical formoterol 65 ± 3.7, $n=20$, $0.05>P>0.01$; Mann Whitney U-test) and the vessels were less densely labelled with carbon. There was a correlation between the plasma exudation response (µl) and the number of carbon-labelled vessels in those animals in which both responses were measured (Spearman's correlation coefficient = 0.415, 95% confidence interval 0.181 – 0.604, $P<0.001$).

Discussion

In this study reproducible plasma exudation responses to intraluminally (tracheal lumen) administered bradykinin have been demonstrated using a novel preparation in which a segment of trachea was perfused *in vivo* in anaesthetized, spontaneously breathing rats. Formoterol, a highly selective β_2-adrenoceptor agonist caused a marked inhibition, but not abolition, of these exudation responses. The degree of inhibition of formoterol was not clearly dose-related but the duration of inhibition increased in relation to the administered dose of either topical (superfused over the tracheal mucosal surface) or parenterally administered (i.p.) formoterol.

Bradykinin, a potent pro-inflammatory mediator, has been found in increased levels in asthmatic luminal fluids, e.g. sputum and bronchoalveolar lavage fluid (Christiansen *et al.*, 1987; 1992) and it is likely to be generated from high molecular weight plasma kininogens (Proud & Kaplan, 1988). Proteins from the plasma exudate have also been found in the airway lumen of asthmatics (Newcomb & Devald, 1969). The balance of current evidence suggests that bradykinin causes exudation of plasma via a direct activation of specific B_2 receptors for bradykinin on postcapillary venular endothelial cells. This causes discrete contraction of the cells, exposure of their basement membrane and a transient, spontaneously reversible, loss of the barrier function of the endothelium to plasma (gap formation). Bradykinin is also a potent activator of pulmonary c-fibres but selective depletion of tachykinins, by capsaicin pretreatment, has no effect on postcapillary venular gap formation in rats, as shown by the degree of labelling of these microvessels with monastral blue, a particulate tracer (Sulakvelidze & McDonald, 1994); this would exclude effects of bradykinin secondary to a neurogenic inflammation.

The most probable mechanism by which formoterol suppresses bradykinin-induced exudation is a β-adrenoceptor-controlled reduction in either the number of endothelial gaps and/or the mean time during which such gaps remain open and allow exudation of unfiltered plasma from the microcirculation. Observations made in this study that are consistent with the above interpretation include the transient nature of the plasma exudation response, the correlation between formoterol-induced inhibition of the plasma exudation response and the intensity of colloidal carbon labelling of microvessels, and the antagonism of the formoterol response by propranolol. Studies by others have demonstrated gap formation in postcapillary and collecting venules, but not in capillaries or arterioles, in the tracheal mucosa of rats after i.v. bradykinin (Sulakvelidze & McDonald, 1994). Inhibition of substance P-induced plasma exudation has been associated with a reduction in the mean number, but not size, of endothelial gaps in the microvessels in rat trachea (Baluk & McDonald, 1994).

It is possible, but unlikely, that material(s) other than plasma protein were detected by the fluorescence method employed in this study to quantify the amount of plasma-derived protein in the tracheal lumen. 5-Hydroxytryptamine released from mast cells in rat airways and/or secretory proteins produced from the epithelium, e.g. goblet cell-derived mucins, might have contributed to the measured fluorescence of samples. However, formoterol is an exceptionally potent and efficacious functional antagonist of lung mast cell degranulation (Mita & Shida, 1983) and this would prevent release of biogenic amines. There is a sparsity of goblet cells in the tracheal epithelium of healthy rats (Hayashi & Huber, 1977) and the intensity of fluorescence of the rat secretory proteins or mucins, at the excitation wavelength used in the present study, is very low (Miller-Larsson & Brattsand, 1991). Furthermore, as part of another study we have used polyacrylamide gel electrophoresis to analyse the proteins present in the luminal fluid and found no evidence for proteins other than those attributable to plasma.

Although formoterol markedly reduced plasma exudation neither this, nor the colloidal carbon labelling of microvessels,

was completely inhibited, even when very high systemic doses of formoterol were administered or when formoterol was combined with a glucocorticosteroid. This suggests that the functional antagonism exerted by formoterol on microvascular endothelial gap formation cannot fully surmount the contractile processes that are responsible for the gap formation (Baluk & McDonald, 1994). At the doses used in this study, formoterol almost certainly exerted its pharmacological effect via activation of β_2-adrenoceptors (Anderson, 1993) although the increase in the plasma exudation seen in animals treated with propranolol alone (the control group) confounded interpretation of the effects of propranolol on formoterol. The potentiation effect of propranolol, which has previously been reported in non-airway microvascular beds (Svensjö *et al.*, 1977; Svensjö & Roempke, 1985) suggests that endogenous catecholamines may physiologically regulate the permeability of the postcapillary venular endothelium.

Although formoterol did not show a dose-related response (inhibition of plasma exudation), the duration of the response was clearly dose-related. After parenteral (i.p.) administration of formoterol, its effects lasted at least 150 min, a time that most probably reflects the elimination half time of the drug. The results of the studies with superfusion of formoterol onto the tracheal mucosa, where the duration of action of formoterol was at least 120 min, raise more interesting issues. The very high binding affinity of formoterol for β_2-adrenoceptors has allowed its rate of dissociation to be accurately calculated and it is slow compared to short-acting adrenoceptor agonists (Lemoine, 1992). However, slow dissociation from the adrenoceptors alone could not account for the above observations on the duration of action of topical formoterol, since low and high doses of drug which produce the same degree of functional antagonism of gap formation should have the same duration of action. A possible reason for the disparity is that formoterol may partition into the lipid membrane of the cell in a concentration-proportional manner and form a local depot within a given tissue. This could extend the effective duration of action of the drug (Anderson *et al.*, 1994).

Erjefält & Persson (1991) reported a sustained anti-exudative effect of formoterol after its instillation into the tracheal airways but, in contrast to the present study, formoterol remained in continuous contact with the mucosa during the elicited response. Jeppsson *et al.* (1994) have recently observed an extended duration of action for formoterol, and also for salmeterol (another lipophilic, long-acting and highly β_2-selective adrenoceptor agonist) but not for terbutaline, when administered by aerosol to guinea-pig isolated perfused lungs *in vitro*. In the present study, a sustained effect was seen for formoterol applied directly onto the tracheal mucosa in total doses that were within the range expected to be in the major airways after a single inhalation of the recommended therapeutic dose of 12 μg (Löfdahl & Svedmyr, 1989; Kerrijbin, 1990; Anderson *et al.*, 1994). These observations suggest that the inhaled route enables these drugs to access the bronchial circulation, a critical determinant of their effects on microvascular permeability.

In conclusion, the results of the present study indicate that selective β_2-adrenoceptor activation by formoterol produces a sustained and marked, but incomplete, inhibition of bradykinin-induced plasma extravasation in a novel, anatomically-defined perfused airway segment model *in vivo*. This inhibitory effect of formoterol was not associated with abolition of microvascular permeability, as determined by colloidal carbon deposition in the microvessels. It is recognised that plasma extravasation may be an important pathological process in human asthma and that its suppression may therefore be of potential therapeutic benefit. However the β_2-adrenoceptor agonist formoterol, even when administered in conjunction with glucocorticosteroid, caused incomplete suppression of either microvascular permeability or plasma exudation. There is also a lack of comparative information on the rat and human bronchial mucosal microcirculations. In light of this, the results of this study in rats should be treated with a degree of circumspection in relation to interpreting whether formoterol exerts effects other than bronchodilatation when used in human asthma.

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