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H_1- and H_2-receptor characterization in the tracheal circulation of sheep

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1 The effects of histamine, the specific H_1-agonist SKF 71481-A_2 and the H_2-agonist dimaprit were examined on tracheal vascular resistance in sheep anaesthetized with pentobarbitone. Tracheal vascular resistance was determined by perfusing the cranial tracheal arteries at constant flows and measuring inflow pressures. Changes in tracheal smooth muscle tone were also measured.

2 Histamine and SKF 71481-A_2 contracted the tracheal smooth muscle and this effect was blocked by the H_1-antagonist mepyramine. Stimulation of H_2-receptors with dimaprit had no effect on tracheal smooth muscle tone.

3 Histamine had a complex action on the tracheal vasculature producing either a triphasic change (early dilatation then constriction followed by late dilatation) or just a constriction. SKF 71481-A_2 always produced a biphasic change in vascular resistance (dilatation followed by constriction). Dimaprit dilated the tracheal vasculature.

4 The late dilatation produced by histamine in some sheep was blocked by bilateral cervical vagotomy but the mechanism for this effect is not known. No other responses to histamine, SKF 71481-A_2 or dimaprit were affected by vagotomy.

5 The vasoconstriction produced by histamine and SKF 71481-A_2 was antagonized by mepyramine indicating a H_1-receptor-mediated effect. Cimetidine had no effect on the vasoconstriction to histamine suggesting a lack of involvement of H_2-receptors.

6 The vasodilatation produced by histamine and SKF 71481-A_2 was also antagonized by mepyramine, again suggesting a H_1-receptor-mediated action. Cimetidine had no effect on the vasodilator response to histamine indicating no involvement of H_2-receptors in this response.

7 The dilator effect of dimaprit was antagonized by cimetidine suggesting this effect was mediated by H_2-receptors.

8 We conclude that H_1-receptors in the various parts of the sheep tracheal vasculature can cause increases and decreases in total tracheal vascular resistance; that H_2-receptors decrease resistance; and that the tracheal smooth muscle contracts on activation of H_1-receptors but has no response to H_2-agonists.

Introduction

The tracheobronchial circulation plays a major role in providing nutrition to the lower airways. Bronchial and tracheal vessels may also control the clearance of chemical mediators from the airways, regulate the development of airway wall oedema and control heat exchange in the tracheobronchial tree (Baier *et al.*, 1985).

Histamine is a mediator of airway anaphylaxis and has various effects on the airways including bronchoconstriction (mediated by H_1-receptors; White *et al.*, 1987), increased microvascular permeability (Brigham & Owen, 1975), mucus secretion (Webber & Widdicombe, 1987) and vasoconstriction of the pulmonary circulation (H_1-receptors; Tucker *et al.*, 1975; Ahmed *et al.*, 1982). Histamine also affects the tracheobronchial circulation; it increases bronchial blood flow in sheep (Zapata-Ortiz *et al.*, 1967) and dogs (Bruner & Schmidt, 1947; Yanaura *et al.*, 1981) when injected directly into the bronchial artery. Histamine administered as an aerosol also increases bronchial blood flow in sheep and this action of histamine on bronchial arterioles may be mediated by H_2-receptors (Long *et al.*, 1985).

Studies of the pharmacology of the tracheobron-

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chial circulation including the action of histamine have been largely restricted to the bronchial vessels. However, interpretation of these studies is complicated because the majority of the bronchial circulation drains via bronchopulmonary anastomoses into the pulmonary circulation. Anatomically the tracheal circulation is simpler than that of the bronchi, lacking 'tracheopulmonary anastomoses' (Laitinen *et al.*, 1987a).

The tracheal vasculature in dogs is under direct neural control (Laitinen *et al.*, 1987b) and can be modified by reflexes from the heart and lungs (Sahin *et al.*, 1987). There are also a large number of chemical mediators which can affect the resistance of tracheal vessels, including agonists at muscarinic cholinoceptors and α- and β-adrenoceptors (Laitinen *et al.*, 1987c), inflammatory mediators (Laitinen *et al.*, 1987d) and a large number of neuropeptides (Laitinen *et al.*, 1987e; Salonen *et al.*, 1988). Histamine dilates the tracheal vasculature in dogs and this effect is dose-related (Laitinen *et al.*, 1987c). However, in view of the considerable species variation in the responses to histamine of airway smooth muscle (Simonsson & Svedmyr, 1981) and pulmonary vessels (Shirai *et al.*, 1987), we have examined the effect of this mediator on the tracheal circulation in sheep using the method previously described for dogs (Laitinen *et al.*, 1987b). We have also investigated the receptor mechanisms which mediate the actions of histamine and of specific H_1- and H_2-agonists on the tracheal circulation.

Methods

Experiments were carried out with seven sheep (body weight 33.4 $\pm$ 1.3 kg, mean $\pm$ s.e.mean) of either sex. All sheep were anaesthetized with sodium pentobarbitone (initially 20 mg kg^{-1}, i.v.). Additional anaesthetic was given as required to maintain surgical anaesthesia. Body temperature was monitored with a rectal thermometer and was maintained between 38 and 40°C without supplementary heating sources. Both femoral arteries were catheterised (8 FG, Portex). One catheter was connected to a pressure transducer (P23Db, Gould) for the measurement of systemic arterial blood pressure; the other was used to supply blood to the tracheal perfusion circuit. A femoral venous catheter (8 FG, Portex) was inserted for administration of heparin and supplemental doses of anaesthetic.

A low cervical tracheostomy was performed and a tracheal cannula was inserted and connected to a Fleisch pneumotachograph to give airflow. All animals were paralysed with an intravenous injection of gallamine triethiodide (1 mg kg^{-1}) and ventilated with a tidal volume of 12–16 ml kg^{-1} at a constant rate of 28 breaths per min. After paralysis, additional doses of anaesthetic were given at the same rate as before injection of gallamine. Furthermore, when the animal showed signs of spontaneous breathing a supplementary dose of gallamine was given and this was always accompanied by a dose of anaesthetic. Also, if an irregular blood pressure trace was observed an additional dose of pentobarbitone was given. These procedures ensured that the paralysed animals were well anaesthetized during the experiment.

Measurement of tracheal vascular resistance

The preparation of the tracheal arteries was similar to that previously described for greyhound dogs (Laitinen *et al.*, 1987a). However, in the dog the trachea is perfused mainly via the branch of the superior thyroid artery which can be seen to supply the cranial trachea. In sheep, the superior thyroid artery is small and has very small or no apparent branches to the trachea. Therefore, arteries lower in the neck were chosen for perfusion of the trachea.

The common carotid arteries were exposed on both sides of the trachea. A single artery was isolated coming from the carotid artery in the middle of the neck. This artery divided into several branches going to trachea, skeletal muscle and glandular tissue. As far as possible all branches which did not supply the trachea were tied off. On the opposite side of the trachea to where the branching artery was found, one or two much smaller arteries which did not branch were isolated coming from the carotid artery and travelling dorsally to the trachea. Other arteries were found coming from the carotid in this region which did not supply the trachea and these were tied off. In five out of seven sheep the large branching artery came from the left carotid artery whereas in the other two sheep it came from the right.

Catheters (8 FG, Portex) were inserted orthogradely into both common carotid arteries below the arteries supplying the trachea. These catheters were used for connection to the perfusion circuit and for local administration of drugs. The common carotid arteries were tied off cranial to the tracheal arteries. To ensure an intact blood supply to the brain and a normal pressure in the carotid sinuses, the occluding catheters in the common carotid arteries on both sides were by-passed with plastic tube loops.

Perfusions of the tracheal wall on both sides were with blood from a reservoir filled from the femoral artery, at constant rates by two peristaltic pumps (MHRE Mk 4, Watson-Marlow). The perfusion pressures were measured by two pressure trans-

ducers (P23ID, Gould). Each perfusion flow rate was adjusted so that tracheal perfusion pressure was close to systemic arterial pressure. All sheep were given heparin (50,000 u, i.v.) before perfusion and the distribution of perfused circulation was tested by close-arterial injection of Evans Blue. In all sheep the main branching artery perfused the mucosa on both sides of the trachea and the length of trachea perfused covered between five and ten cartilaginous rings. On the opposite side, the arteries going dorsally to the trachea perfused a very small region of the visible lateral trachea (about 1 cm × 3 cm) but may have perfused a larger region of the dorsum of the trachea; a little dye also appeared in adjacent tissues such as the oesophagus. Tracheal vascular resistance was calculated from measurements of tracheal artery pressure at constant perfusion flow. Inflow pressure was divided by flow to give vascular resistance.

Measurement of tracheal smooth muscle tone

Changes in tracheal smooth muscle tone were measured by recording changes in the external diameter of the cranial trachea. To determine external diameter, a fixed bar was placed on one side of the trachea and a thin lever connected to a force-displacement transducer (FT03C, Grass) was gently placed on the other side using a micromanipulator (Figure 1). Changes in force were read against a standard calibration curve to give the displacement of the lever tip (range ± 0.6 mm) indicating a change in external tracheal diameter.

Addition of agonists

Injections of histamine, SKF 71481-A_2 (2-(2-aminoethyl) thiazole dihydrochloride) and dimaprit were given directly into the arterial catheter supplying the tracheal vascular bed, in 0.1 or 0.2 ml of isotonic saline. All injections were given to the left perfusion circuit for sheep whose trachea was perfused mainly from the left and to the right perfusion circuit for sheep where the perfusion was mainly from the right. Drugs were injected at intervals of at least 10 min, always allowing for complete recovery from the previous injection.

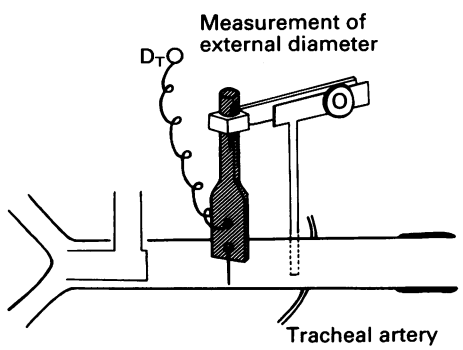

Figure 1 Diagram of the device used to measure external tracheal diameter.

Between four and seven doses of each agonist were given singly and in random order to obtain dose-response curves. Only peak changes in perfusion pressures and tracheal diameter were measured.

Controls were carried out as injections of 0.1 or 0.2 ml of saline into the perfusion circuit. These caused brief increases in pressure during the injection (5–10 s), presumably distending the segment from which pressure was recorded, followed by small transient (10–20 s) decreases in perfusion pressure, presumably due to a lowering of blood viscosity (Figures 2, 6 and 8). Peak changes due to drugs always occurred and were measured at times after the effects of viscosity had elapsed.

Antagonist studies

In sheep where histamine had a triphasic effect on tracheal vascular resistance, two control doses of histamine were chosen for the antagonist studies. The first dose produced 60–80% of the maximum early and late dilator phases of the response, and only a very small (10–20% of maximum) constrictor response, whereas the second larger dose produced near maximum dilator responses and 60–80% of maximum constrictor effect. In all other cases a single dose of each agonist was chosen which had produced approximately 60–80% of the maximum change in vascular resistance for that agonist.

Control doses of histamine, dimaprit and SKF 71481-A_2 were given singly and in a random order both before and after bilateral vagotomy, then before and after cimetidine (250 mg, i.v.) and finally before and after mepyramine (50 mg, i.v.). At least 5 min was allowed after injection with cimetidine or mepyramine before the addition of a dose of agonist. The duration of action of cimetidine was monitored by its antagonism of the vasodilatation produced by dimaprit and was found to be maintained for at least 45 min after i.v. injections. Neither cimetidine (250 mg, i.v.) nor mepyramine (50 mg, i.v.) had any significant effect on tracheal vascular resistance, tracheal diameter or systemic blood pressure.

Evaluation of results

The log dose-response curves in Results were obtained by pooling the results from all experiments and were plotted by hand. All results are given as means ± s.e.mean. The pD_2 values (where pD_2 =

$-\log ED_{50}$ and ED_{50} is the dose producing 50% of the maximum response to an agonist) and maximum responses were estimated from log dose-response curves plotted for each agonist from each single experiment and the mean values were calculated. The log dose-response curves from these single experiments were fitted to the data points by a computerised, non-linear, least squares estimate (Marquardt, 1963). The effects of vagotomy and the antagonists on control responses to the agonists were analysed for statistical significance by Student's *t* test for paired values.

Drugs

Histamine dihydrochloride and Evans Blue were obtained from Sigma, and heparin sodium from CP Pharmaceuticals. The following drugs were kindly donated by the companies indicated: cimetidine, dimaprit and SKF 71481-A_2 (Smith Kline and French); mepyramine maleate, sodium pentobarbitone (Sagatal) and gallamine triethiodide (May and Baker).

Results

Responses to histamine

In four of seven sheep, histamine (5–100 nmol) had a triphasic effect on tracheal vascular resistance (Figure 2a). An initial rapid dilatation of the tracheal vascular bed (maximum between 25 and 30 s) was followed by a constriction (50–60 s) and then, much later, a further dilatation (3–5 min). All three phases of the histamine response were dose-dependent (Figure 3a). The computer-estimated pD_2 values for the early and late phase dilatations were 8.1 ± 0.4 and 7.8 ± 0.3, respectively, and the maximum % changes in tracheal vascular resistance were -16.1 ± 4.1 and -30.8 ± 6.4%, respectively. It was not possible to estimate a pD_2 value or maximum response for the constrictor phase of the histamine effect because not enough doses of histamine were used for the computer to fit a dose-response curve to the data points.

In three of seven sheep, histamine (0.5–100 nmol) produced only an increase in tracheal vascular resistance (Figure 2b) which was dose-dependent (Figure 3b). The pD_2 value for this constriction was 7.8 ± 0.2 and the maximum increase in tracheal vascular resistance was $+54.6 \pm 7.2$%.

In all seven sheep histamine (5–100 nmol) produced a decrease in the diameter of the trachea (Figure 2a and b) which was dose-dependent (Figure 4) and presumably represented smooth muscle contraction. The pD_2 value for this effect was 7.9 ± 0.4 ($n = 7$) and the maximum reduction in tracheal diameter was -0.28 ± 0.05 mm ($n = 7$) compared to a total tracheal diameter of 12.2 ± 1.6 mm ($n = 7$). Histamine had no significant effect on systemic arterial blood pressure at any of the doses studied.

Responses to SKF 71481-A_2 and dimaprit

In the five sheep studied the specific H_1-agonist SKF 71481-A_2 (50–500 nmol) had a biphasic effect on the tracheal vasculature (Figure 5a). An initial dilatation (maximum between 25–30 s) was followed by a later constriction (40–60 s). Both the dilator and constrictor effects were dose-dependent (Figure 6a) and at each dose of SKF 71481-A_2 the dilatation was approximately twice as great as the constriction. The pD_2 values for the dilatation and constriction were 7.1 ± 0.3 and 6.9 ± 0.4, respectively and the maximum changes in tracheal vascular resistance were -23.6 ± 0.8 and $+14.2 \pm 1.8$%, respectively. SKF 71481-A_2 (50–500 nmol) decreased the diameter of the trachea in a dose-dependent manner indicating contraction of the tracheal smooth muscle (Figures 4 and 5a). However, it was not possible to estimate a pD_2 value or maximum response for this effect.

In each of six sheep the H_2-agonist dimaprit (0.1–5 μmol) decreased tracheal vascular resistance (Figure 5b). This dilator effect was dose-dependent (Figure 6b) with a pD_2 value of 6.4 ± 0.2 and a maximum response of -18.4 ± 3.8%. Dimaprit (0.1–5 μmol) produced no significant change in the external diameter of the trachea suggesting no effect on tracheal smooth muscle tone. Neither SKF 71481-A_2 (50–500 nmol) nor dimaprit (0.1–5 μmol) had any significant effect on systemic blood pressure.

Antagonism of the response to histamine

In sheep which showed a triphasic response to histamine, the control dose chosen for the antagonism studies (see Methods) produced changes in tracheal vascular resistance for the early dilatation, constriction and later dilatation of -15.7 ± 1.2, $+7.4 \pm 2.0$ and -21.7 ± 2.4%, respectively. The early dilatation and constriction were not significantly affected ($P > 0.05$) by bilateral cervical vagotomy (-14.1% and $+8.0$%, Figure 7a) but the later dilatation was significantly reduced (-1.6%, $P < 0.05$) by this treatment (Figure 7a). The initial dilator and constrictor responses were not significantly affected by the H_2-antagonist cimetidine (-14.2% and $+6.4$%, Figure 7a) but both were highly significantly reduced ($P < 0.01$) by the H_1-antagonist mepyramine (-1.4 and $+0.8$% respectively, Figure 7a). The late dilator response had been initially abolished by vagotomy, and cime-

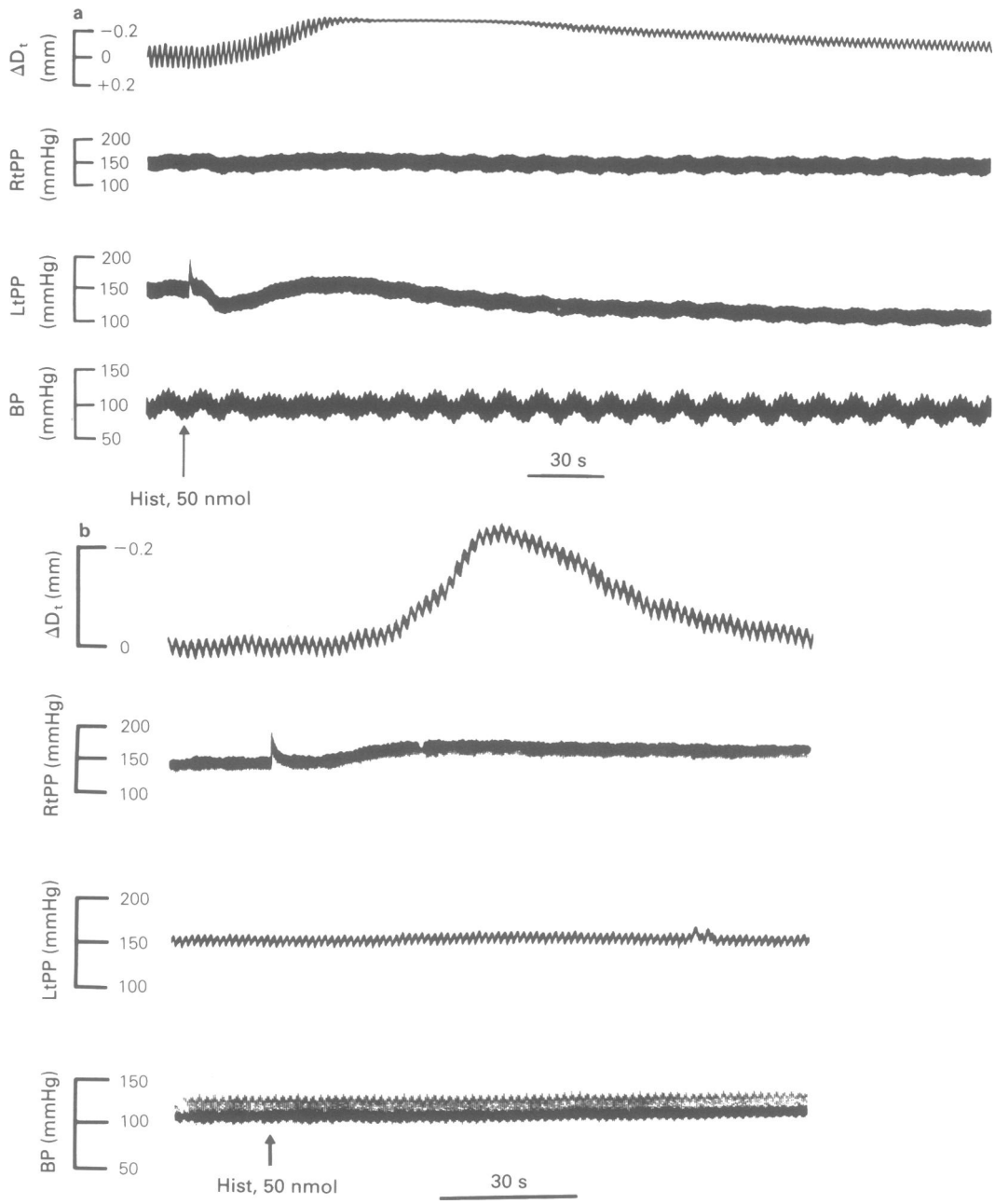

Figure 2 Responses to close arterial injection of histamine (Hist, 50 nmol) on (a) the left and (b) right sides. Traces from above down: external tracheal diameter (D_t), right arterial perfusion pressure (RtPP), left perfusion pressure (LtPP) and systemic arterial blood pressure (BP). The transient rises in left arterial perfusion pressure in (a) and right arterial perfusion pressure in (b) immediately after the injection are pressure artefacts due to the injection. Subsequently in (a), histamine causes a triphasic change in left tracheal perfusion pressure; a fall in perfusion pressure (15 s) is followed by a rise (50 s) and a much later fall (4 min). There is also a decrease in tracheal diameter representing smooth muscle contraction. In (b), histamine causes a rise in right tracheal perfusion pressure and a decrease in tracheal diameter. Contralateral effects were very small and blood pressure did not change.

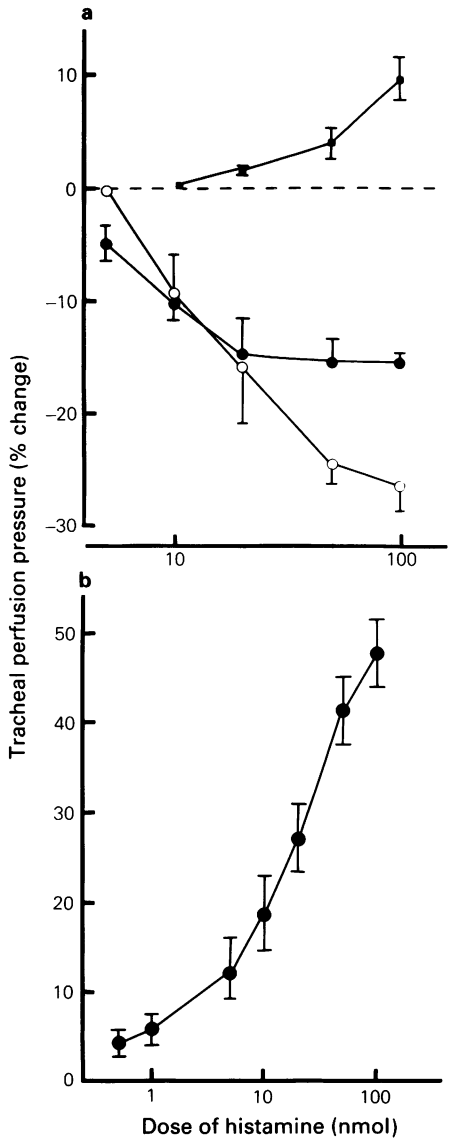

Figure 3 Dose-response curves for (a) the triphasic effect of histamine on tracheal vascular resistance. (●) Early dilatation (maximum at 20 s), (■) constriction (50–60 s), (○) late dilatation (3–5 min). (b) The constrictor effect of histamine on tracheal vascular resistance. Points are means of 3–4 determinations and the vertical lines represent s.e.means.

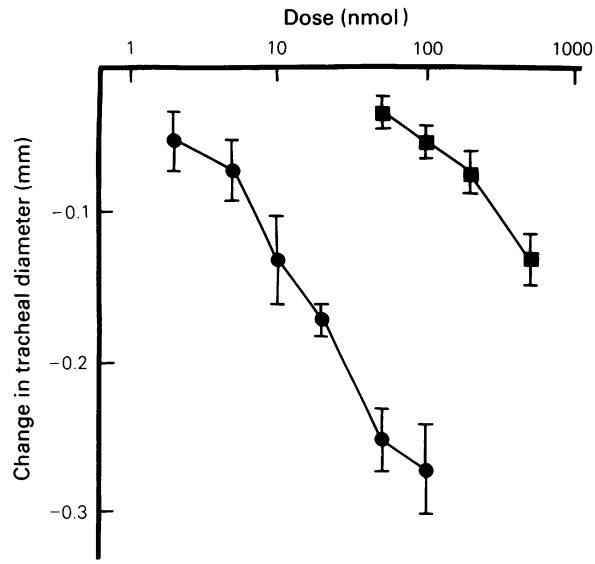

Figure 4 Dose-response curves showing the effect of histamine (●) and SKF 71481-A_2 (■) on external tracheal diameter. Both drugs reduce tracheal diameter indicating smooth muscle contraction. Points are means of 3–7 determinations and the vertical lines represent s.e.means.

tidine had no further action on it. In sheep which showed only a vasoconstriction to histamine the mean control increase in tracheal vascular resistance was +24.6 ± 5.8% (n = 7). This constriction was not significantly affected by vagotomy (+24.5 ± 5.4%, n = 7) or cimetidine (+26.9 ± 6.5%, n = 5) but was highly significantly reduced by mepyramine (+2.6 ± 0.9%, n = 4).

The decrease in tracheal diameter produced by control doses of histamine (−0.23 ± 0.04 mm) was not significantly changed by vagotomy (−0.23 mm) or cimetidine (−0.18 mm) but was highly significantly reduced by mepyramine (−0.004 mm, Figure 8).

Antagonism of responses to SKF 71481-A_2 and dimaprit

The mean changes in tracheal vascular resistance produced by control doses of SKF 71481-A_2 were −15.5 ± 1.8% and +9.1 ± 1.1% for the initial dilatation and constriction, respectively. Neither of these effects was significantly changed by vagotomy (−14.6 and +9.0%, respectively, Figure 7b) or cimetidine (−17.3 and +9.6%, Figure 7b) but both were highly significantly reduced by mepyramine (−2.8 and +1.4%, Figure 7b). The decrease in tracheal diameter produced by control doses of SKF 71481-A_2 (−0.08 ± 0.02 mm) was not significantly changed by vagotomy (−0.10 mm) or cimetidine (−0.10 mm) but was highly significantly reduced by mepyramine (−0.02 mm, Figure 8).

The mean reduction in tracheal vascular resistance

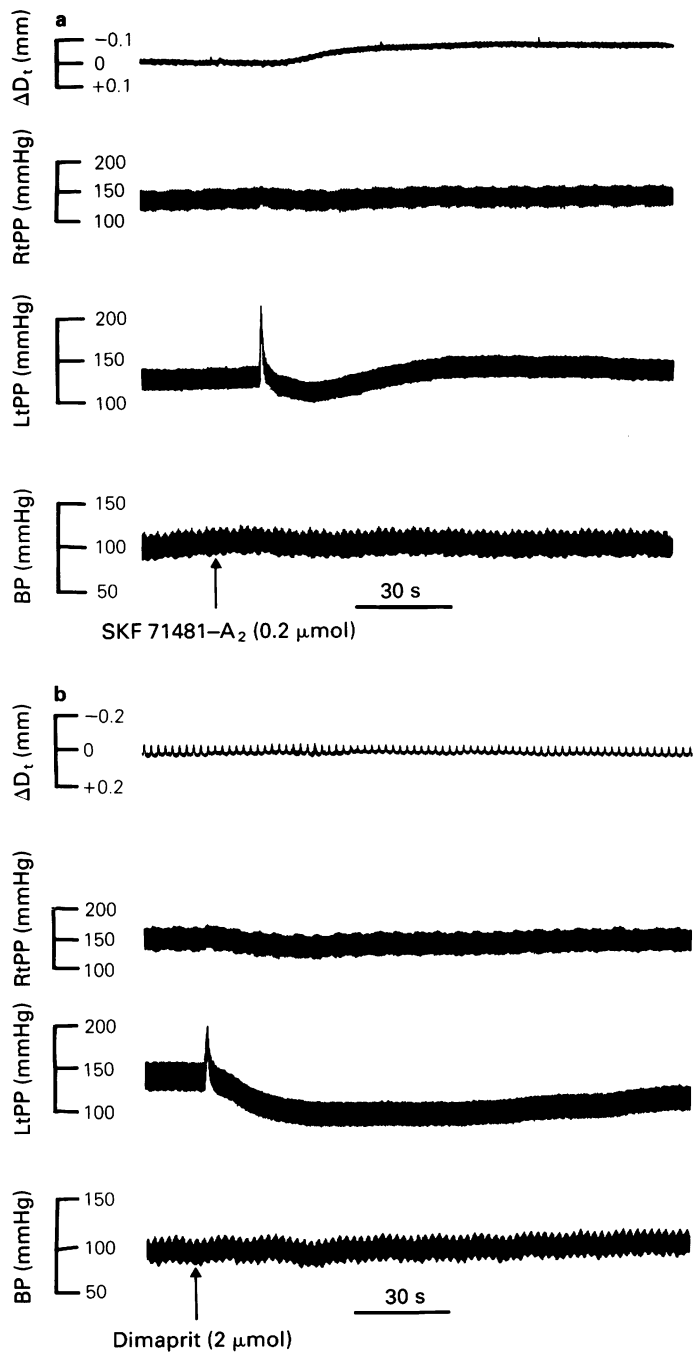

Figure 5 Responses to close-arterial injections of (a) SKF 71481-A_2 (0.2 μmol) and (b) dimaprit (2 μmol) on the left side. Traces and abbreviations are as in Figure 2. In (a), SKF 71481-A_2 produces a biphasic change in left tracheal perfusion pressure; a fall in perfusion pressure (15 s) is followed by a rise (60 s). There is also tracheal smooth muscle contraction. In (b), dimaprit produces a fall in tracheal perfusion pressure with no change in tracheal diameter.

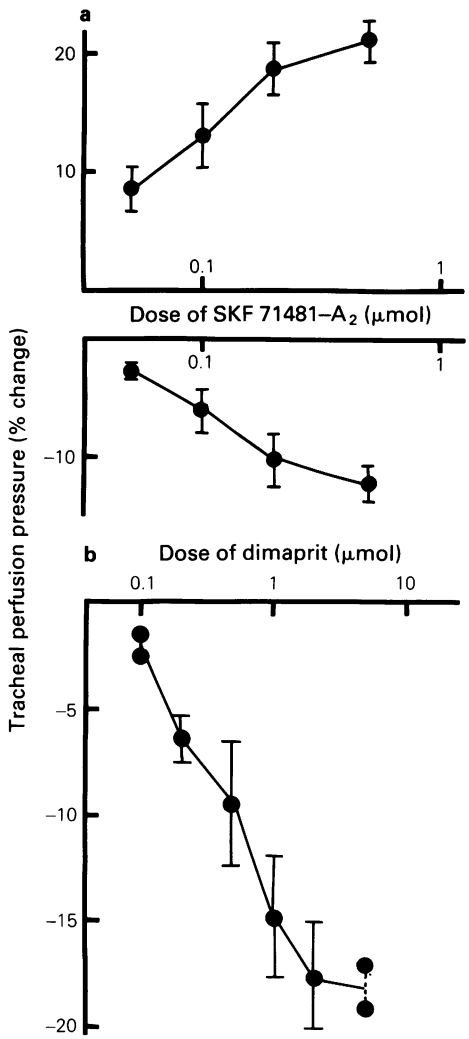

Figure 6 Dose-response curves for (a) the biphasic effect of SKF 71481-A_2 on tracheal vascular resistance. Lower graph–early dilatation (maximum 20s), upper graph–constriction (50–60s). (b) The effect of dimaprit on tracheal vascular resistance. Points are means of 2–7 determinations and the vertical lines represent s.e.means.

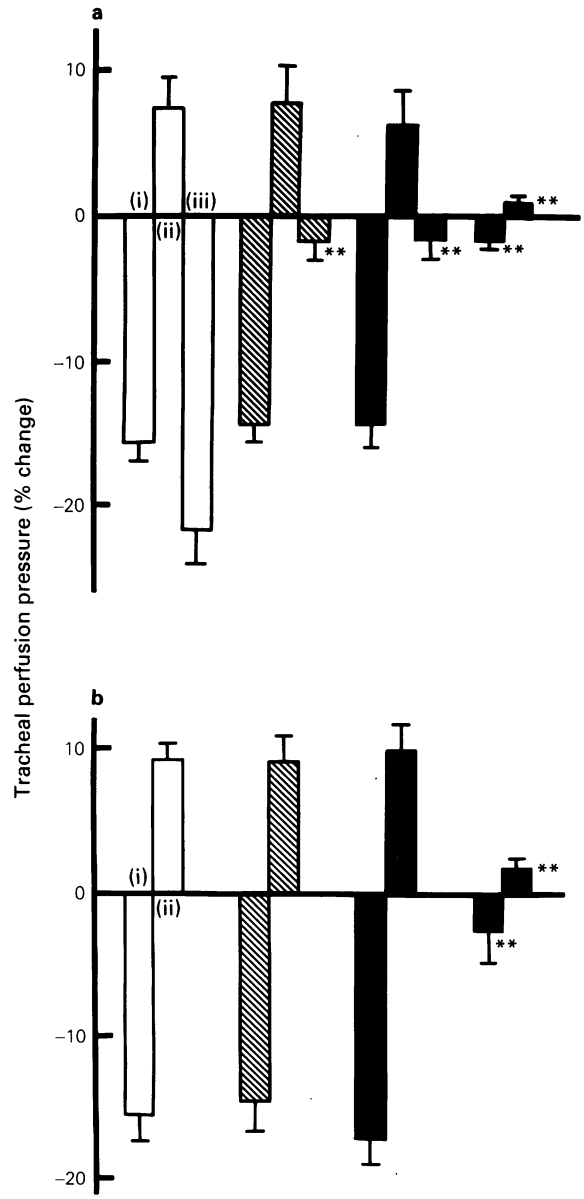

Figure 7 The effect of vagotomy (hatched columns), cimetidine (250mg, i.v., stippled columns) and mepyramine (50mg i.v.; solid columns) on (a) control early dilatation (i), constriction (ii) and late dilatation (iii) induced by histamine and (b) control early dilatation (i) and constriction (ii) induced by SKF 71481-A_2. The control doses were chosen and the protocol followed as described in Methods. The results are the means of 5–11 determinations with s.e.mean shown by vertical lines. ** Highly significantly different ($P < 0.01$) from control value (open columns) by Student's t test for paired observations.

produced by control doses of dimaprit ($-16.0 \pm 2.2\%$, $n = 8$) was not significantly changed by vagotomy ($-15.1 \pm 1.6\%$, $n = 8$) but was significantly reduced by cimetidine ($-2.2 \pm 0.9\%$, $n = 7$).

Discussion

Histamine and SKF 71481-A_2 reduced the external diameter of the trachea, suggesting contraction of

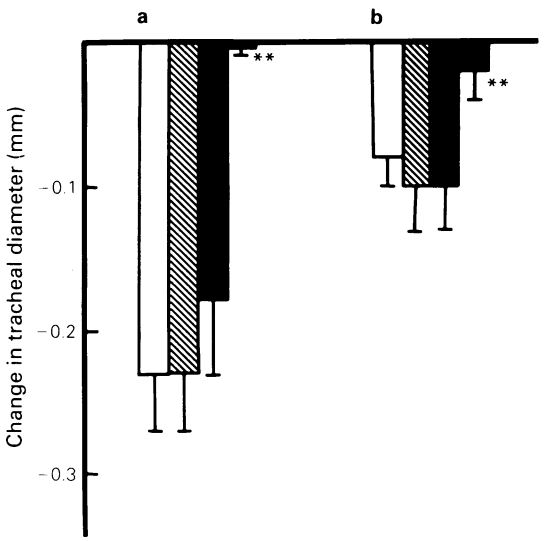

Figure 8 The effect of vagotomy (hatched columns), cimetidine (250 mg i.v., stippled columns) and mepyramine (50 mg, i.v.; solid columns) on control tracheal smooth muscle contractions produced by (a) histamine and (b) SKF 71481-A_2. The control doses were chosen and the protocol followed as described in Methods. The results are the means of 4–7 determinations with s.e.mean shown b vertical lines. **Highly significantly different ($P < 0.01$) from control vaue (open columns) by Student's test for paired observations.

tracheal smooth muscle. Histamine was approximately 50 times more potent than SKF 71481-A_2 in this respect, although it was not possible to determine the maximum effect of SKF 71481-A_2. In contrast, histamine was found to be only 5 times more potent than SKF 71481-A_2 on guinea-pig tracheal spirals (Harrison *et al.*, 1984) and was equipotent with SKF 71481-A_2 on guinea-pig tracheal strips (Duncan *et al.*, 1980). The action of histamine and SKF 71481-A_2 on tracheal smooth muscle was antagonized by mepyramine suggesting a H_1-receptor-mediated effect, which is consistent with numerous other studies on airway smooth muscle preparations from many species including sheep (Eyre, 1969; Ahmed *et al.*, 1980). The contractile effect of histamine was not affected by bilateral cervical vagotomy suggesting that histamine was not causing smooth muscle contraction by a reflex action via irritant or C-fibre receptors (Coleridge & Coleridge, 1986). The smooth muscle contraction produced by histamine was also not affected by the H_2-antagonist cimetidine suggesting that histamine was having no effect on H_2-receptors on the smooth muscle in this preparation. Others have found that the contractile action of histamine on airway smooth muscle *in vitro* and *in vivo* can be enhanced by the addition of an H_2-antagonist, suggesting that histamine can stimulate H_1- and H_2-receptors on the airway smooth muscle simultaneously in humans (Dunlop & Smith, 1977), guinea-pigs (Okapko *et al.*, 1978) and sheep (Ahmed *et al.*, 1980), the latter being relaxant.

A lack of effect of H_2-receptor stimulation on tracheal smooth muscle tone was also shown using the specific H_2-agonist dimaprit which induced no change in the tracheal diameter even at high doses, although the muscle could relax to reflex actions such as lung inflation. A similar lack of effect of dimaprit on the basal tone of guinea-pig lung strips has previously been found (Foreman *et al.*, 1985).

The effect of histamine on the sheep tracheal vasculature was complex and varied between sheep. Responses to histamine fell into two categories; in four of seven sheep a triphasic change in vascular resistance was observed with histamine whereas in the other three sheep only a constriction was seen. The responses of the tracheal vasculature to specific H_1- and H_2-agonists were more consistent. SKF 71481-A_2 produced a dilatation followed by a constriction in all sheep and dimaprit only a dilatation in all sheep; therefore the late dilatation due to histamine may be mediated by H_2-receptors. This possibility was not tested, since in the sheep that had a triphasic response to histamine the late dilatation was abolished by bilateral cervical vagotomy, suggesting that the effect depended on tonic activity in the vagus nerve. However, the time to reach maximum response was between 3 and 5 min which is unlikely to be a reflex action on C-fibres or irritant receptors in either the trachea or the lungs (Coleridge & Coleridge, 1986). Furthermore, there was no change in systemic blood pressure, which is reduced when bronchial C-fibres are stimulated. However, it is possible that histamine may, via a reflex action, release other slowly acting vasoactive substances locally in the tracheal tissue. Some neuropeptides (vasoactive intestinal peptide and calcitonin gene related peptide) cause long lasting dilatation of the canine tracheal vasculature (Salonen *et al.*, 1988). Since vagotomy was always performed before the addition of H_1- and H_2-antagonists it was not possible to see what effect these antagonists would have on the late dilatation.

The small vasoconstriction seen in sheep which had a triphasic response to histamine and the vasoconstriction produced by histamine in all other sheep were both antagonized by mepyramine, suggesting that these vasoconstrictions are mediated by H_1-receptors. Cimetidine had no effect on these responses suggesting no involvement of H_2-receptors. The specific H_1-agonist SKF 71481-A_2

also constricted the tracheal vascular bed, and this response was blocked by mepyramine. Therefore, it is clear that there is a H_1-receptor-mediated vasoconstriction of the tracheal vascular bed. Histamine produces pulmonary vasoconstriction via H_1-receptors (Tucker *et al.*, 1975; Ahmed & King, 1986), but a H_1-receptor-mediated vasoconstriction of the systemic tracheal vasculature is an unusual response since histamine reduces systemic arterial blood pressure in dogs and sheep (Tucker *et al.*, 1975; Ahmed *et al.*, 1982), dilates the bronchial artery of the sheep (Long *et al.*, 1985; Link *et al.*, 1985) and dilates the tracheal vasculature in dogs (Laitinen *et al.*, 1987c,d).

The initial vasodilatation observed with histamine in four of seven sheep and the initial vasodilatation to SKF 71481-A_2 were both antagonized by mepyramine, suggesting that these effects were also mediated by H_1-receptors. However, it is not clear why histamine failed to induce a vasodilatation in three sheep, particularly since SKF 71481-A_2 did cause vasodilatation in these sheep. The vasoconstrictor action of histamine in these sheep was very large and may have masked any initial dilator effect.

The initial vasodilatation induced by histamine was not affected by the H_2-antagonist cimetidine, suggesting no involvement of H_2-receptors in this response. In contrast, the histamine-induced reduction in systemic arterial blood pressure (Ahmed & King, 1986) and the dilatation of the bronchial artery in sheep (Long *et al.*, 1985) are both mediated by H_2-receptors. Moreover, the H_2-agonist dimaprit can produce a dose-dependent vasodilatation of the tracheal vascular bed. Therefore, stimulation of both H_1- and H_2-receptors can dilate the tracheal vascular bed in sheep. It is not known why both receptor types can mediate vasodilatation or why stimulation of H_1-receptors can result in vasodilatation followed by vasoconstriction. More than one tissue (trachea and oesophagus) may be perfused and, therefore, more than one vascular bed may be responding to the agonists. However, this is unlikely since the extent of perfusion was tested by injection of Evans Blue dye (see Methods), which indicated that the trachea was the main site of perfusion. It is possible that part of the vascular response to histamine could derive from mechanical effects as the respiratory smooth muscle contracts. However, this is unlikely since the smooth muscle can contract whilst the vasculature dilates (e.g. with methacholine) and contracts (e.g. with histamine). Furthermore, structural studies of the sheep tracheal circulation (Goulding *et al.*, 1988) have shown that most of the blood flow to the trachea is mucosal and not associated with smooth muscle, so contraction of the smooth muscle is unlikely to have any significant effect on the vasculature. Another possibility is that different vessels in the vascular bed contain different populations of H_1- and H_2-receptors which will be stimulated differently depending on which agonist is used. For example, arterioles and venules may have different numbers of H_1- and H_2-receptors, and in addition the sheep has a system of 'venous' sinuses, the effect of which on total vascular resistance is not known (Goulding *et al.*, 1988). However, our results indicate what would happen to total tracheal vascular resistance when H_1- and H_2-receptors are stimulated irrespective of where the histamine-receptor agonists act. The complexity of action of histamine on the tracheal vasculature is reflected by that on the pulmonary vasculature. Thus, in the cat, histamine constricts pulmonary arteries with internal diameter of between 300 and 400 μM and dilates pulmonary arteries with internal diameter less than 200 μM. The small pulmonary veins are also constricted (Shirai *et al.*, 1987). Therefore, in preparations containing a mixture of different histamine-sensitive vessels, histamine must have a very complex action.

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