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Effect of vagotomy on airway hyperreactivity to endogenously released neurotransmitters at 18–24 h after inhaled antigen

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Abstract

Airway reactivity was examined in anaesthetized guinea-pigs 18–24 h after inhalation challenge of ovalbumin-sensitized animals with ovalbumin. Bronchoconstrictor responses were measured from the increases in pulmonary inflation pressure. The study was undertaken to examine whether ovalbumin challenge induced airway hyperreactivity to neurotransmitters released endogenously by vagal nerve stimulation. Stimulation parameters were selected to cause release of either acetylcholine (0.3 ms pulse width for 3 s, 20 V, 2–40 Hz), both acetylcholine and neuropeptide (5 ms pulse width for 15 s, 20 V, 0.5–8 Hz) or neuropeptide only, using the latter parameters in the presence of atropine. The vagi were paired for stimulation and in some experiments were cut central to the stimulation point. Frequency–response curves for acetylcholine- and neuropeptide-mediated bronchoconstrictor responses to vagal stimulation when the nerves were intact revealed no airway hyperreactivity after ovalbumin challenge. The presence of atropine failed to reveal airway hyperreactivity. However, when the vagi were cut, the frequency–response curves were displaced to the left after ovalbumin challenge compared with saline challenged animals, indicating airway hyperreactivity. This airway hyperreactivity was significant after atropine and suggests an increase in sensitivity to endogenously released neuropeptides rather than acetylcholine. It also indicates that the airway hyperreactivity is dependent on removal of the afferent vagal pathways. Frequency–response curves for cholinergic stimulation (0.3 ms) with intact vagi revealed no airway hyperreactivity after antigen challenge. Comparisons of exogenously administered 5-hydroxytryptamine (5-HT, 300 ng/100 g i.v.) and a single vagal stimulation of 0.3 ms pulse width (cholinergic) revealed no airway hyperreactivity to either stimulus after ovalbumin challenge. However if the vagi were cut, airway hyperreactivity was observed, again suggesting that removal of afferent pathways is important for revealing airway hyperreactivity in the anaesthetized guinea-pig. Ovalbumin challenge caused significant increases in the bronchoconstrictor responses to a single dose of capsaicin (50 μg/100 g i.v.) or dose–response curves to bradykinin. Since these agents release neuropeptides from sensory C-fibres, this is further support for a raised sensitivity to endogenously released neuropeptides.

Keywords: Airway hyperreactivity; Vagal stimulation; Ovalbumin challenge; Capsaicin; Bradykinin; 5-HT (5-hydroxytryptamine, serotonin); (Guinea-pig, anaesthetized)

1. Introduction

Several autonomic defects of the airways previously thought to be implicated as causative factors in human asthma are now believed to be secondary to the disease or its treatment (Barnes, 1986a). However, there is still considerable evidence that neural mechanisms may contribute to the symptoms of asthma (Barnes and Thomson, 1992). The concept of neurogenic inflammation which is well established in the skin and gut may also apply to the airways (Barnes, 1986b).

Electrical stimulation of guinea-pig vagus nerves in vivo produces a component of bronchoconstriction that is not inhibited by atropine (Andersson and Grundström, 1983). This excitatory non-adrenergic non-cholinergic (NANC) response is thought to be mediated by the retrograde release of neuropeptides from unmyelinated C-fibres. A similar response has been reported in human airways in vitro (Webber, 1990), but this finding is not consistent. Pro-inflammatory neuropeptides have been localised to capsaicin-sensitive sensory nerves (C-fibres) in the airways (Lundberg and Saria, 1987). They have potent effects on many airway functions including bronchomotor tone, secretions, the circulation and inflammatory cells, however, the precise role of each peptide is still not clear. There may

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be several peptides in co-existence, e.g., neurokinin A, substance P and calcitonin gene-related peptide (CGRP) within the same sensory nerve (Uddman and Sundler, 1987). Little is known about the optimum conditions for neuropeptide release, although they are thought to respond to high-frequency firing and therefore may only be co-released with classical neurotransmitters in certain circumstances. Electrical stimulation of the vagus nerves causes acute neurokinin A, substance P and CGRP release from sensory nerves in the lung (Lundberg et al., 1984) and subsequent bronchoconstriction.

When sensitised guinea-pigs are challenged with antigen, the bronchoconstriction which follows is orchestrated by many different factors, e.g., histamine, eicosanoids and platelet-activating factor-acether (PAF) (Barnes et al., 1988). Vargas (1990) found that along with histamine, vagal influence played an important part in the bronchoconstrictor response to antigen during the early response to antigen challenge.

There is abundant evidence for airway hyperreactivity in anaesthetized guinea-pigs to exogenously administered spasmogens at 24 h after an antigen challenge (Coyle et al., 1988; Havill et al., 1990; Sanjar and Morley, 1990; Noonan et al., 1991; Farmer et al., 1992; Howell et al., 1993; Johnson and Broadley, 1995). However, few studies have examined whether airway hyperreactivity to endogenous spasmogens released by electrical stimulation of the vagus nerves occurs after challenge of sensitized guinea-pigs with ovalbumin. Nieri and Daffonchio (1992) investigated the ability of an ovalbumin challenge to increase airway reactivity to electrical stimulation of the vagus in anaesthetised guinea-pigs. Airway reactivity was assessed shortly after saline or ovalbumin challenge and no airway hyperreactivity was seen, despite obtaining airway hyperreactivity to the effects of exogenously applied acetylcholine, substance P and capsaicin. They suggest that the increased reactivity seen with exogenous agonists was not strictly related to enhanced bronchial smooth muscle contraction. Two further studies investigated the possibility of multiple antigen-challenges in inducing hyperresponsiveness. Ballati et al. (1992) found the airways were hyperresponsive to neurokinin A aerosol and to excitatory NANC stimulation, one week after the last antigen-challenge, a time when no difference in responsiveness was seen with aerosol-administered acetylcholine or histamine. They later showed that airway hyperresponsiveness to a selective neurokinin 1 agonist and also to electrical-stimulation of the vagus nerves (mainly NANC) was evident 7 to 9 days after the last antigen-challenge and was linked to the airway inflammation which was also maximal at this time (Perretti et al., 1995).

To date, no published work has examined airway reactivity to electrical stimulation of the vagus nerves at the time of the late phase bronchoconstriction in guinea-pigs, 18 to 24 h after a single ovalbumin challenge (Lewis and Broadley, 1995). In the present study, we examine the responses to vagal stimulation in anaesthetized guinea-pigs under conditions in which cholinergic or NANC neurotransmisssion or both occurs.

2. Materials and methods

2.1. Sensitisation and challenge with ovalbumin

Male Dunkin–Hartley guinea-pigs (Halls, Staffordshire, UK) weighing 350 to 400 g were sensitised to ovalbumin according to the method described by Andersson (1980). Briefly, guinea-pigs received (10 μg) and $Al(OH)_3$ (100 mg) in 1 ml normal saline, by a single intraperitoneal injection.

At 14 to 21 days after sensitisation, the animals underwent a macroshock challenge by exposure to a nebulised solution of ovalbumin (1% w/v in normal saline for 2 min) in a sealed perspex box ($350 \times 200 \times 150$ mm). The aerosol was generated by a Wright nebuliser using medical air and the atmosphere in the exposure box was allowed to become saturated with the aerosol for 2 min before introducing the animal. Mepyramine (10 mg/kg i.p.) was given 30 min beforehand to protect against fatal anaphylaxis. In spite of the mepyramine cover, animals still experienced a severe anaphylactic reaction manifest as exaggerated inhalations and/or cough usually with some degree of cyanosis and, rarely, convulsions. Those animals not responding to antigen-challenge with apparent bronchoconstriction were excluded from the study. Verification of sensitisation was also obtained by giving an intravenous bolus of ovalbumin (40 μg) after the determination of airway reactivity under general anaesthesia.

Controls were sensitised animals which were treated in an identical manner but were given nebulised normal saline instead of an ovalbumin challenge.

2.2. Determination of airway reactivity

The anaesthetised guinea-pig model was used for the assessment of airway calibre. Animals were taken 18 to 24 h after ovalbumin challenge and anaesthetised with sodium pentobarbitone (60 mg/kg) by intraperitoneal injection and placed in the dorsal recumbent position on a heated small animal operating table. A cannula was inserted into the trachea and the lungs mechanically ventilated by a Harvard constant volume respiration pump (58 strokes/min of 1 ml air per 100 g body weight). Pulmonary inflation pressure was measured from a lateral port in the afferent limb of the ventilator circuit. Systemic arterial blood pressure measurements were made via a polythene catheter placed in the right carotid artery. The right jugular vein was also cannulated for intravenous ovalbumin administration (to verify sensitisation at the end of each experiment). Vagus nerves were prepared by blunt dissection (at cervical level) and in some animals were cut then tied together.

The caudal portion was stimulated using a square-wave stimulator (SRI). The nerves were kept moist throughout the experiment by application of saline.

The choice of stimulation pattern was based on those used in the study by Nieri and Daffonchio (1992). For release of endogenous acetylcholine only, stimulation was at 0.3 ms pulse width for 3 s at 20 V, while for release of endogenous neuropeptide and acetylcholine, stimulation was at 5 ms pulse width at 20 V for 15 s. Atropine was also used in some experiments (1 mg/kg i.v., 15 min before frequency–response curves were commenced) in order to reveal the neuropeptide component of bronchoconstriction when stimulated using the latter parameters. A period of equilibration, typically 10 min, was allowed after surgical intervention. During this time the lungs were hyperinflated with 3 tidal volumes of room air (by occluding outflow) and allowed to return to basal values. Frequency–response curves were then obtained.

In some experiments, the effect of a single stimulation train (0.3 ms pulse width, 20 Hz, 20 V) for 3 s was obtained and compared in the same animal with the effect of a single bolus dose of 5-hydroxytryptamine (5-HT, 300 ng/100 g i.v.). The effects of bradykinin were examined by intravenous administration of increasing bolus doses, allowing approximately 3 min between doses or until the pulmonary inflation pressure had returned to baseline value. The effect of a single bolus dose of capsaicin was also examined in saline and ovalbumin-challenged animals.

2.3. Data analysis

Pulmonary inflation pressure, which is known to increase approximately in proportion to the degree of bronchoconstriction (at constant volume) was measured. Baseline values were typically 10–13 cmH_2O (which was approximately 30% of maximum effect of vagal nerve stimulation) and were not statistically different between treatment groups. The peak pulmonary inflation pressure was measured after nerve stimulation or an intravenous dose of spasmogen. For dose– or frequency–response curves, this was expressed as a percentage of the maximum pulmonary inflation pressure determined before each experiment by manual occlusion of the tracheal cannula. The arithmetic mean ± S.E.M. percentage maximum pulmonary inflation pressure values were calculated. Non-cumulative log frequency– (according to the method of Nieri and Daffonchio, 1992) or log dose–response curves were constructed from the mean percentage maximum pulmonary inflation pressure values. From individual frequency– or dose–response curves, the effective frequency or dose to double baseline value (EF_2 or ED_2) was calculated. Geometric mean EF_2 or ED_2 values were determined (together with their 95% confidence limits) and were analysed using the Student's unpaired *t*-test to determine whether the reactivity was significantly different between control and ovalbumin-challenged animals. In experiments where a single stimulation or bolus dose was used, the increase in pulmonary inflation pressure above baseline was calculated and the arithmetic mean ± S.E.M. determined. A probability level of $P < 0.05$ was considered statistically significant in all experiments.

2.4. Blood pressure measurement

Changes in diastolic blood pressure were monitored for control and ovalbumin-challenged guinea-pigs in all experiments. In this way it was possible to ensure that the nerves were viable as indicated by a fall in blood pressure on stimulation. Responses also provided a means of monitoring the effect of the anaesthetic on vagally-mediated responses. Guinea-pigs not exhibiting blood pressure responses were excluded from the study.

To compare the blood pressure effects of vagal nerve stimulation or spasmogens in control and ovalbumin-challenged animals, the frequency or dose of spasmogen required to cause a fall in diastolic pressure of 10 mmHg (EF_{10} or EC_{10} value) was calculated. These values were then compared using the Student's *t*-test.

2.5. Drugs

Atropine sulphate, bradykinin, capsaicin, heparin sodium (porcine), 5-hydroxytryptamine (creatinine sulphate complex, 5-HT) and ovalbumin were all purchased from Sigma (Poole, Dorset, UK). Sagatal (pentobarbitone sodium B.P. 60 mg/ml) was purchased from Rhône Mérieux (UK). Aluminium hydroxide and mepyramine maleate were gifts from Rhône Poulenc-Rorer (Dagenham, Essex, UK).

3. Results

3.1. Vagal stimulation at 5 ms to release acetylcholine and / or neuropeptide

Electrical stimulation using 5 ms pulse width (both neuropeptide- and acetylcholine-mediated) of the both cut and intact vagal nerves (in separate experiments) caused frequency-dependent bronchoconstrictions. Differences in the bronchoconstrictor response to vagal stimulation determined at 18 to 24 h after an ovalbumin or saline challenge are compared in Table 1 and Fig. 1. Ovalbumin-challenge did not enhance the airway reactivity to electrical vagal stimulation in animals with intact vagus nerves (Fig. 1A) and the presence of atropine had no effect on this result (Fig. 1C). However, in animals with cut vagi (Fig. 1B and D), the frequency–response curve was shifted to the left and this was significant in atropine treated animals. Thus, ovalbumin-induced airway hyperreactivity was evident in vagotomised, atropine treated guinea-pigs.

The effect of cutting the vagus nerves per se on the frequency–response curves can be seen in Table 1. Electri-

Table 1
Airway reactivity to vagal stimulation (5 ms, 20 V, 15 s) in sensitised, anaesthetised guinea-pigs determined 18–24 h after saline or ovalbumin inhalation challenge

Conditions	Mean EF_2, (Hz) (95% confidence limits)				
	Saline	*n*	Ovalbumin	*n*	*P* value
Intact vagus	1.4 (1.1–1.9)	10	1.5 (1.1–2.2)	9	0.86
Intact vagus with atropine	4.6 (3.0–7.1)	8	4.1 (2.7–6.4)	8	0.73
Cut vagus	5.2 (2.1–12.7)[b]	4	1.7 (0.7–4.3)	4	0.09
Cut vagus with atropine	9.8 (7.3–13.1)[b]	7	4.9 (3.9–6.1)	8	0.004[a]

Hyperreactivity is defined as a significant leftward shift of the frequency–response curve and is denoted by [a]($P < 0.05$).
Significant reduction in sensitivity due to vagotomy compared with the intact vagus is denoted by [b]($P < 0.05$).

cal stimulation of the vagus nerves produced significantly less bronchoconstriction in vagotomised, saline-challenged guinea-pigs than in animals with intact vagi ($P = 0.036$). In the presence of atropine, which by itself reduced the response to vagal stimulation, cutting the vagus nerves caused a further significant ($P = 0.019$) reduction of the response to vagal stimulation. In ovalbumin-challenged guinea-pigs, however, cutting the vagus nerves failed to significantly modify the sensitivity to vagal nerve stimulation both in the absence ($P = 0.521$) and presence ($P = 0.809$) of atropine.

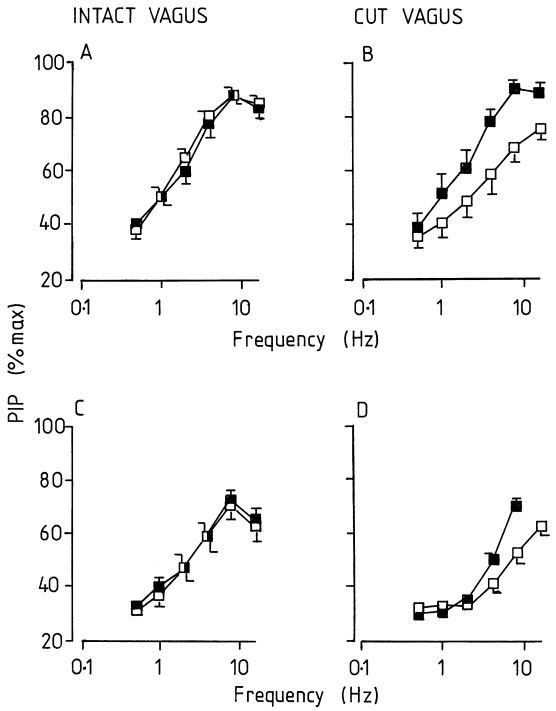

Fig. 1. The effect of saline (□) or ovalbumin challenge (■) 18–24 h beforehand on frequency–response curves for the bronchoconstrictor responses to electrical stimulation of the vagus nerves in anaesthetised guinea-pigs. Stimulation was at 5 ms pulse width for 15 s to release both acetylcholine and neuropeptide. Stimulation was performed with intact nerves in the absence (A, $n = 10,9$) or presence (C, $n = 4,4$) of atropine (1 mg/kg i.v.). In separate animals the effect of ovalbumin challenge was examined on nerve stimulation after cutting the vagi above the point of stimulation, again in the absence (B, $n = 8,8$) or presence of atropine (D, $n = 7,8$). Mean responses (±S.E.M.) are shown, measured as the peak pulmonary inflation pressure (PIP) and expressed as the minimum possible PIP.

3.2. Vagal stimulation at 0.3 ms to release acetylcholine

Vagal nerve stimulation with pulses of 0.3 ms duration (cholinergic) produced frequency-dependent bronchoconstrictor responses when the vagus was intact (Fig. 2). There was no significant difference between the curves obtained at 18–24 h after saline and ovalbumin challenge.

3.3. Effects of spasmogens

When the effects of a single dose of 5-HT (300 ng/100 g i.v.) and a single stimulation (20 Hz, 20 V) with 0.3 ms trains (cholinergic) were compared in the same guinea-pigs with intact vagi, there was no hyperreactivity. The bronchoconstrictions produced by 5-HT and electrical stimulation, measured as the increase in pulmonary inflation pressure, did not differ significantly between saline and ovalbumin-challenged animals ($P > 0.05$, Fig. 3). After atropine treatment, there was still no significant increase in the response to either 5-HT or electrical stimulation in ovalbumin-challenged guinea-pigs compared with saline controls. In contrast, when the vagi were cut, the responses to 5-HT ($P = 0.05$) and electrical stimulation ($P < 0.05$) were significantly potentiated by the ovalbumin challenge (Fig. 3). Similarly, after atropine, the bronchoconstriction by 5-HT ($P = 0.004$) and the very small residual response to electrical stimulation ($P < 0.05$) were significantly potentiated by the ovalbumin challenge.

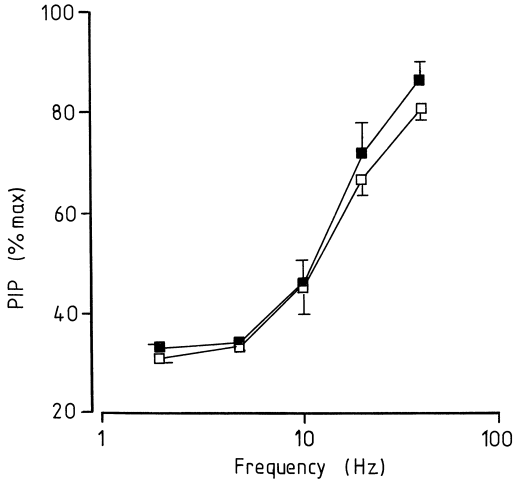

Fig. 2. The effect of saline (□, $n = 4$) or ovalbumin challenge (■, $n = 4$) 18–24 h beforehand on frequency–response curves for the bronchoconsistor responses to electrical stimulation of the vagus nerves in anaesthetised guinea-pigs. Stimulation was at 0.3 ms pulse width for 3 s to release acetylcholine and the nerves were intact. Mean responses (±S.E.M.) are shown, measured as the peak pulmonary inflation pressure (PIP) and expressed as the maximum possible PIP.

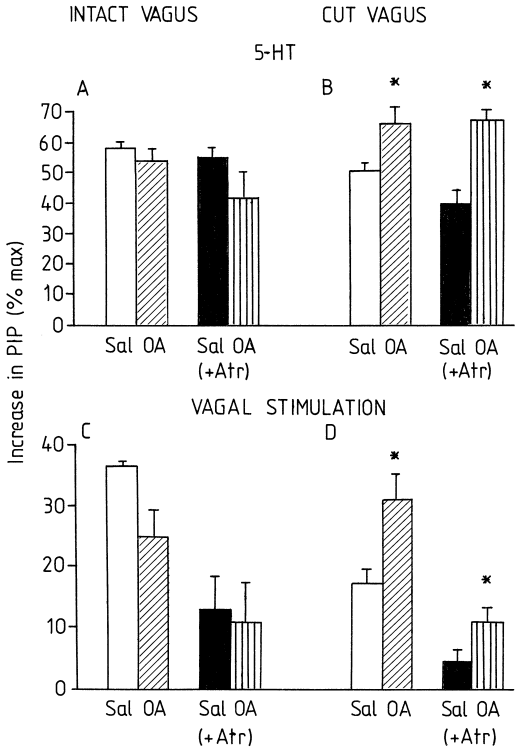

Fig. 3. The effect of saline (Sal) or ovalbumin (OA) challenge 18–24 h beforehand on mean ($n = 4$) bronchoconsistor responses to a single dose of 5-hydroxytryptamine (5-HT) (A and B; 300 ng/100 g i.v.) and, in the same animal, a single stimulation of the vagus nerve (C and D) using parameters to release acetylcholine (0.3 ms, 20 Hz, 20 V for 3 s). Responses were obtained with the vagus nerves intact (A and C) or with cut vagi (B and D) either in the absence (saline, open bar; ovalbumin, diagonal hatched bar) or presence (saline, solid bar; ovalbumin, vertical hatching) of atropine (+ Atr; 1 mg/kg i.v.). Mean responses (±S.E.M.) are shown, measured as the increase in peak pulmonary inflation pressure (PIP) above the basal level and expressed as a percentage of the maximum possible increase in PIP.

Ovalbumin-challenged guinea-pigs receiving an intravenous bolus of capsaicin (50 μg/100 g) experienced a significantly greater degree of bronchoconstriction ($P = 0.045$) than saline-challenged animals (78.0 ± 8.5% and 32.3 ± 10.2%, respectively, $n = 4$ and 4). Bradykinin produced a dose-related increase in pulmonary inflation pressure but the maximum dose administered failed to produce a maximum effect (Fig. 4). In ovalbumin-challenged animals, the dose–response curve was raised above that for the controls. The ED_2 value in ovalbumin-challenged animals (0.71(0.23–2.22) μg/100 g) was significantly ($P = 0.03$) less than after saline challenge (4.86(3.3–7.06) μg/100 g).

3.4. Effects of vagal stimulation on blood pressure

Vagal stimulation produced a fall in diastolic blood pressure, which was similar in control and ovalbumin-challenged guinea-pigs. Fig. 5 shows the falls in blood pressure after vagal nerve stimulation to encourage cholinergic

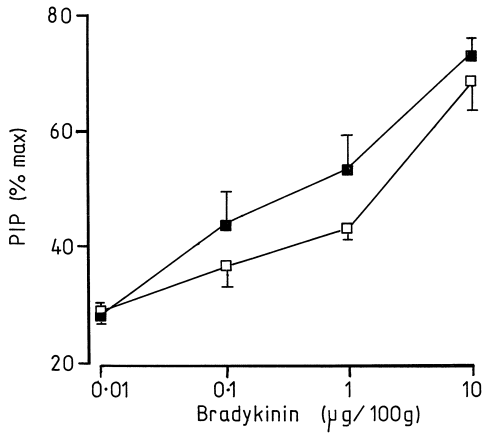

Fig. 4. The effect of saline (□, $n = 6$) or ovalbumin challenge (■, $n = 6$) 18–24 h beforehand on the bronchoconsistor responses to increasing intravenous doses of bradykinin in anaesthetised guinea-pigs. Mean responses (±S.E.M.) are shown, measured as the peak pulmonary inflation pressure (PIP) and expressed as the maximum possible PIP.

(0.3 ms, 20 V, for 3 s; intact nerves) or peptidergic transmission (5 ms, 20 V for 15 s; bilateral vagotomy; in the presence of atropine). There were no significant differences in the EF_{10} values between control and ovalbumin-

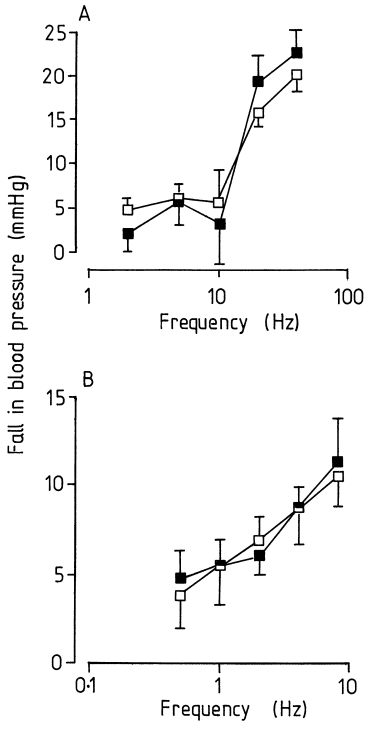

Fig. 5. Mean (±S.E.M.) falls in diastolic blood pressure of anaesthetised guinea-pigs in response to increasing frequencies of vagal stimulation. Sensitised guinea-pigs were exposed to inhalation challenge with saline (□) or ovalbumin (■) 18–24 h beforehand. In (A) ($n = 4,4$) the stimulation parameters favoured acetylcholine release (0.3 ms, 20 V for 3 s) and the vagi were intact. In (B) ($n = 7,8$) the stimulation conditions were for peptidergic transmission (5 ms, 20 V for 15 s) with the nerves cut and atropine present (1 mg/kg i.v.).

challenged animals after cholinergic (15.6 ± 2.9 and 15.2 ± 3.0 Hz, respectively, $P = 0.93$, $n = 4$ and 4) or peptidergic type nerve stimulation (7.0 ± 1.4 and 6.1 ± 1.1 Hz, respectively, $P = 0.66$, $n = 7$ and 8).

4. Discussion

Reactivity of the airways to endogenously released neurotransmitter was assessed at 18–24 h after an ovalbumin challenge of sensitised guinea-pigs. At this time after an ovalbumin challenge, we have previously demonstrated the appearance of a late phase bronchoconstriction in the conscious guinea-pig (Lewis et al., 1996), although in this study the direct effects of the ovalbumin challenge on airway function were not recorded. We have also shown a small but significant airway hyperreactivity in the anaesthetized guinea-pig at this time after the ovalbumin challenge to a limited number of exogenously administered spamogens, including the inhaled thromboxanemimetic, U-46619 (Johnson and Broadley, 1997). It can therefore be assumed that the ovalbumin challenge conditions and the anaesthetized guinea-pig model were appropriate for the induction of and detection of airway hyperreactivity. In the present study, these conditions were used to measure the bronchoconstrictor responses to vagal nerve stimulation in anaesthetized guinea-pigs employing stimulation parameters that favour cholinergic or peptidergic neurotransmitter release or both. When the parameters of electrical stimulation were aimed at acetylcholine and peptide release (5 ms pulse width), there was no hyperreactivity after ovalbumin challenge.

When atropine was administered to remove the cholinergic component so that the response to vagal stimulation was presumed to involve mainly release of peptides, again there was no hyperreactivity to vagal stimulation. No experiments were performed to confirm that neuropeptides were responsible for this bronchoconstriction; we based our assumptions on the work of Nieri and Daffonchio (1992) and could find no further information in the literature. However, it has been shown that the excitatory NANC-mediated contractile response to electrical stimulation of guinea-pig isolated trachea in the presence of atropine (Charette et al., 1994) and of isolated perfused lung (Lou et al., 1993) are due to the release of neurokinin A acting on NK_2 receptors. Although the dose of atropine used was sufficient to block a supra-maximal dose of exogenous methacholine (unpublished) through its action on post-junctional muscarinic M_3-receptors on airway smooth muscle, atropine will also block pre-junctional muscarinic M_2-receptors with equal efficacy (Barnes and Thomson, 1992). Muscarinic receptors of the M_2-subtype are autoinhibitory, their blockade resulting in greater endogenous acetylcholine release which may overcome the muscarinic M_3 receptor blockade and thus tending to enhance the effect of vagal stimulation and overcome the post-junctional blockade. Indeed, there was a residual bronchoconstriction to the cholinergic stimulation (0.3 ms) after atropine (Fig. 3D). Under the conditions favouring acetylcholine release (0.3 ms pulse width), no airway hyperreactivity was seen to vagal stimulation. On this evidence it appears unlikely that airway hyperreactivity occurs to the released acetylcholine.

When the animals were vagotomised, increased airway reactivity was observed to vagal stimulation (5 ms) favouring either both transmitters or neuropeptide (in the presence of atropine). This ovalbumin-induced hyperreactivity was significant in animals also treated with atropine. In contrast to the present results, Nieri and Daffonchio (1992) found no hyperreactivity to vagal stimulation using either stimulation conditions and with the vagi cut, but they evaluated the sensitivity at only 1 h after the ovalbumin challenge.

Section of the vagus nerves above the point of stimulation decreased the airway response to vagal stimulation in saline-challenged animals. This suggests that activation of centrally directed afferent pathways were involved in the normal bronchoconstriction to vagal stimulation. Interestingly, however, no loss of bronchoconstrictor potency was seen after vagotomy in the ovalbumin-challenged animals. It therefore appears that in ovalbumin-challenged animals the central pathways are not involved in the bronchoconstrictor response to vagal stimulation. Thus, ovalbumin challenge somehow counteracts or compensates for the loss of responsiveness associated with removal of central pathways. In asthma, damage to the airway epithelium exposes C-fibre afferent nerve endings and stimulation of these nerves by inflammatory mediators (e.g., bradykinin) may result in local reflexes with antidromic conduction down afferent nerve collaterals (Barnes, 1986b). These local or axon reflexes lead to excitatory NANC bronchoconstriction due to the release of neuropeptides (e.g., substance P, neurokinin A and CGRP) from sensory nerves. This bronchoconstriction is mediated by the action of neurokinin A (and to a lesser extent substance P) on neurokinin NK_2-receptors in the airway (Lou et al., 1993). It is possible that inflammation of the airways secondary to ovalbumin-challenge could lead to increased neuropeptide release by local reflexes. This theory is consistent with results seen in the current work, i.e., increased nervous activity due to local reflexes compensates for the loss of central bronchoconstrictor pathways in ovalbumin- but not saline-challenged animals. As a result, there is no net change in response to vagal stimulation when the vagi are cut. Endogenous tachykinins are also thought to be important in toluene diisocyanate-induced airway hyperreactivity in guinea-pigs, an effect which is not due to their known effect on airway vascular permeability (Thompson et al., 1987). Thus, airway hyperreactivity to vagal stimulation only occurred when the vagi were cut and was significant when atropine was present to inhibit the cholinergic component. This would suggest an increased reactivity to

neuropeptide rather than to acetylcholine. To test this hypothesis, the effects of capsaicin (Maggi and Meli, 1988) and bradykinin (Kaufman et al., 1980), agents known to cause bronchoconstriction by stimulation of sensory C-fibres which induces the antidromic release of neuropeptides, were examined.

When capsaicin was given as an intravenous bolus, despite a high degree of inter-animal variation, the constrictor responses were significantly greater in ovalbumin-challenged animals than controls. Similarly, dose–response curves for the bronchoconstrictor responses to bradykinin were shifted upwards indicating an increase in airway reactivity. These results suggest that there was an increase in the sensitivity to endogenously released peptides.

Several drugs effective in the clinical treatment of asthma are known to modulate the release of neuropeptides from airway sensory nerves, e.g., sodium cromoglycate inhibits bronchial C-fibre reflex activity (Dixon et al., 1979) as does the more potent, related compound, nedocromil sodium (Verleden et al., 1990). The beneficial effects of these drugs and possibly the corticosteroids, could be partially due to their inhibitory effect on axon reflex mechanisms (Barnes, 1986b). Dexamethasone has been shown to inhibit ozone-induced increases in airway reactivity to exogenous and endogenous neuropeptides (Murlas et al., 1993).

To determine sensitivity to spasmogens acting at other receptor sites, a single dose of 5-HT was injected intravenously in animals that also received a single vagal stimulation to release acetylcholine (0.3 ms). With intact vagus nerves, there was no hyperresponsiveness to either 5-HT or vagal stimulation after ovalbumin challenge either in the absence or presence of atropine. However, when the vagi were cut, there was an increase in sensitivity to both 5-HT and vagal stimulation. This is consistent with our findings using frequency–response curves. The hyperresponsiveness of the airways was therefore also evident to a non-peptide spasmogen. This agrees with other studies, including our own (Johnson and Broadley, 1995) which have demonstrated hyperreactivity to intravenous spasmogens in anaesthetised guinea-pigs 24 h after ovalbumin challenge (Coyle et al., 1988; Havill et al., 1990; Sanjar and Morley, 1990; Farmer et al., 1992; Howell et al., 1993), including 5-HT (Noonan et al., 1991). Although a component of the bronchoconstriction to 5-HT may involve a direct contraction of airways smooth muscle via 5-HT_2 receptors, there is also probably an indirect component through activation of efferent pathways (Hahn et al., 1978). This is indicated by a reduction of the response by atropine in control animals with cut vagi and by a previous study from this laboratory in conscious guinea-pigs (Lewis and Broadley, 1995).

The results of this study show that airway hyperreactivity to vagal stimulation can be demonstrated at 18–24 h after antigen challenge in anaesthetised guinea-pigs, a time when we have shown the presence of a late-phase bronchoconstriction (Lewis et al., 1996). However, it is only apparent when the vagus nerves are cut and is more evident in the presence of atropine to block muscarinic receptors. The reasons for this remain to be determined but suggest that the vagus nerves and the cholinergic component may be involved in antigen-induced airway hyperreactivity.

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