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AN *IN VITRO* STUDY OF THE PROPERTIES OF SINGLE VAGAL AFFERENTS INNERVATING GUINEA-PIG AIRWAYS

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SUMMARY

1. A novel preparation of the trachea and main bronchi with attached vagus nerve from the guinea-pig maintained *in vitro* was used to study the properties of single vagal afferent nerve fibres with identified receptive fields.

2. Recordings were made from twenty-eight C fibres with a mean conduction velocity of $0{\cdot}9 \pm 0{\cdot}1$ m s^{-1} and twenty-four Aδ fibres with a mean conduction velocity of $8{\cdot}4 \pm 1{\cdot}3$ m s^{-1}. Receptive fields for C and Aδ fibres were of small diameter, distributed throughout the trachea and right bronchus and possessed very low mechanical thresholds of $2{\cdot}2 \pm 0{\cdot}4$ and $1{\cdot}1 \pm 0{\cdot}3$ mN respectively.

3. The chemosensitivity of isolated afferents was studied by applying drugs directly onto identified receptive fields. Aδ fibres were insensitive to capsaicin (up to 3 μM), bradykinin (3 μM), histamine (10 μM) and 5-hydroxytryptamine (5-HT; 10 μM) applied for up to 1 min. Histamine (10 μM), 5-HT (10 μM) and *m*-chlorophenylbiguanide (10 μM) were also ineffective in exciting C fibres.

4. Capsaicin, at concentrations ranging from 30 nM to 3 μM, evoked a sustained firing of all C fibres tested when applied for a period of 30 s directly onto receptive fields. Bradykinin (0·1–1 μM) also potently excited C fibres in a concentration-related manner. The effect of bradykinin appeared to be mediated by a B_2 receptor since it was not mimicked by the selective B_1 receptor agonist [des-Arg^9]-bradykinin (3 μM) and was abolished by prior application of the selective B_2 receptor antagonist D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (HOE 140; 0·1 μM). HOE 140 was without effect against capsaicin-evoked discharge of C fibres.

5. Capsaicin- and bradykinin-evoked discharge of C fibres was present to a similar degree in preparations pretreated with ibuprofen (1 μM), indicating that it was not dependent on, or influenced by, endogenous prostaglandin production.

6. These data demonstrate that single vagal afferents may be studied *in vitro* and provide the first examination of the properties of sensory fibres innervating guinea-pig airways. C and Aδ fibres both exhibit low threshold mechanical sensitivity but show marked differences in terms of their chemosensitivity.

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INTRODUCTION

There is increasing evidence that sensory nerves play an important pathophysiological role in airway diseases such as asthma. Several studies have shown that stimulation of vagal sensory fibres either electrically or with the irritant capsaicin produces a variety of effects including bronchoconstriction (Lundberg, Saria, Brodin, Rosell & Folkers, 1983; Forsberg, Karlsson, Theodorsson, Lundberg & Persson, 1988), mucous secretion (Kuo, Rhode, Tokuyama, Barnes & Rogers, 1990), microvascular leakage (Lundberg *et al.* 1983) and cough (Forsberg *et al.* 1988), all of which are characteristic symptoms of inflammatory airway disease. These responses may consist of a central reflex and/or a local axon reflex with release of sensory neuropeptides such as substance P and neurokinin A (Barnes, Baraniuk & Belvisi, 1991). However, it is difficult to demonstrate the possible interaction of inflammatory mediators with sensory nerves using these techniques, since their activation is measured indirectly. Furthermore, these studies cannot provide information regarding the class of sensory fibre which may be stimulated by such mediators.

More direct support for a pathophysiological role of airway sensory nerves comes from studies performed in larger species such as dogs, cats and rabbits, which have utilized the technique of recording from single vagal afferent fibres. These *in vivo* studies have described four classes of afferent fibre ending: slowly adapting stretch receptors and rapidly adapting stretch receptors with myelinated fibres and unmyelinated C fibre endings (Sant'Ambrogio, 1987). The latter group is now subdivided into bronchial and pulmonary C fibre endings (Coleridge & Coleridge, 1977) encompassing the 'deflation', or 'J', receptors initially identified by Paintal (1955, 1969). The stimulation of these sensory receptors may contribute to airway reflexes such as bronchoconstriction, cough and changes in breathing pattern (Coleridge & Coleridge, 1984; Karlsson, Sant'Ambrogio & Widdicombe, 1988). Moreover, rapidly adapting or 'irritant' receptors and C fibre endings have been shown to be stimulated by the inflammatory mediators histamine (Mills, Sellick & Widdicombe, 1969; Coleridge & Coleridge, 1977; Dixon, Jackson & Richards, 1979), bradykinin (Kaufman, Coleridge, Coleridge & Baker, 1980) and prostaglandins (Coleridge, Coleridge, Ginzel, Baker, Banzett & Morrison, 1976).

Whilst *in vivo* studies of this kind have undoubtedly greatly enhanced our understanding of the function of airway sensory receptors, they have a number of possible drawbacks. Firstly, the exact location of receptive fields of isolated single fibres is not easy to define, and whilst myelinated fibres may be detected by their resting discharge and with respiratory manipulations (Widdicombe, 1954; Sant'-Ambrogio, 1987), the study of C fibres is more difficult since they show only a sparse resting discharge with no respiratory modulation (Coleridge & Coleridge, 1984). Secondly, when examining the chemosensitivity of these sensory receptors it is not possible to control the effective concentration of exogenous agents at the receptor site whether they are applied intra-arterially or by inhalation. This is a particular problem when the applied agent is itself vasoactive and thereby influences its own distribution. Finally, it may be difficult to determine whether drugs evoking a discharge in single afferents are acting directly on the fibre ending or indirectly via changes in lung mechanics or the release of some mediators from the vasculature.

The purpose of the present study, therefore, was to examine the properties of single airway sensory fibres *in vitro* where drugs such as inflammatory mediators (histamine, 5-hydroxytryptamine, bradykinin) and selective primary afferent stimulants (capsaicin) can be applied directly onto identified receptive fields of isolated fibres. We have used a novel preparation of the isolated trachea and main bronchi with attached vagus nerve from the guinea-pig, a species widely used for the study of allergic airway inflammation and in which little is known about the properties of vagal afferents. A preliminary account of this work has been published in abstract form (Fox, Barnes, Urban & Dray, 1992).

METHODS

General

Male Dunkin–Hartley guinea-pigs (350–450 g) were killed by anaesthetic overdose (60 mg ml^{-1} intracardial pentobarbitone sodium) and the thorax opened by mid-line incision. The right vagus nerve was sectioned just below the nodose ganglion and removed with the trachea, lungs and oesophagus and placed in modified Krebs solution of the following composition (mM): NaCl, 138·6; KCl, 3·5; $NaHCO_3$, 21·0; Na_2PO_4, 0·58; MgCl, 1·2; $CaCl_2$, 1·5; glucose, 10·0, bubbled with 95% O_2–5% CO_2. The lungs, oesophagus and blood vessels were removed and the vagus nerve cleared of connective tissue. The trachea and main bronchi were then opened longitudinally on their ventral side and pinned, epithelial side up, in a Perspex chamber (dimensions 6·0 × 1·5 × 0·8 cm) and perfused at a rate of 7–8 ml min^{-1} with Krebs solution maintained at 32 °C. The vagus nerve (free length 1·5 cm) was drawn through a small hole into an isolated recording chamber (dimensions 3·5 × 2·0 × 1·0 cm) where it was overlaid with paraffin oil (Fig. 1). Under a dissecting microscope the nerve was desheathed and using fine forceps was teased into small filaments which were further subdivided until single unit activity could be recorded, via a monopolar silver electrode, after stimulation of the main nerve trunk using a silver hook electrode.

Characterization of single fibre properties

After identification of single unit activity, the fibres were characterized as myelinated or unmyelinated according to their conduction velocity calculated from the time and distance (approximately 1 cm) between stimulating and recording electrodes. Receptive fields for individual units were then located by gently probing the surface of the trachea and bronchi with a blunt glass rod, and their mechanical thresholds were determined using calibrated von Frey hairs. The chemosensitivity of single fibres was examined by applying drugs directly onto the receptive fields; agents were perfused using a separate perfusion system at a rate of 2·5 ml min^{-1} into a small plastic tube (internal diameter 2·5 mm) held onto the tissue surface. This perfusion rate was found to provide a rapid transfer of drug without causing undue turbulence which might evoke a mechanical stimulation of the sensory receptor.

A number of agents were tested for their ability to evoke discharges in both myelinated and unmyelinated fibres. Histamine, 5-HT, capsaicin and bradykinin were applied at a range of concentrations for periods of 30 s or 1 min. With individual units at least 20 min was left between drug applications and where more than one concentration of either capsaicin or bradykinin was applied the dose interval was extended to 1 h to avoid desensitization. In all experiments the mechanical sensitivity of receptive fields was checked between each drug application to ensure continued viability of the unit under investigation.

In a separate series of experiments the effects of bradykinin on unmyelinated C fibres were investigated by examining the effects of the selective B_1 receptor agonist [des-Arg^9]-bradykinin ([des-Arg^9]-BK; Regoli & Barabe, 1980), and the selective B_2 receptor antagonist D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (HOE 140; Hock *et al.* 1991). Here a control response in an individual fibre was established to bradykinin and retested 1 h later in the presence of HOE 140 after a prior 5 min perfusion of the receptive field with antagonist. In these same fibres the antagonist was also tested against capsaicin-evoked responses to ensure its selectivity. Finally, responses of C fibres to bradykinin and capsaicin were examined in preparations in which ibuprofen (1 μM) was included in the Krebs solution from the time of the dissection. This concentration of

ibuprofen is sufficient to selectively inhibit cyclo-oxygenase (Moore & Hoult, 1982) and thereby test for possible tissue sensitization by prostaglandins and their involvement in the effects of bradykinin and capsaicin.

Data recording and analysis

Afferent impulses were amplified with an AC amplifier (Neurolog NL104), filtered and monitored using a loudspeaker amplifier and storage oscilloscope. All data were recorded on magnetic tape and analysed off-line using a personal computer with CED 1401 interface (Cambridge Electronic

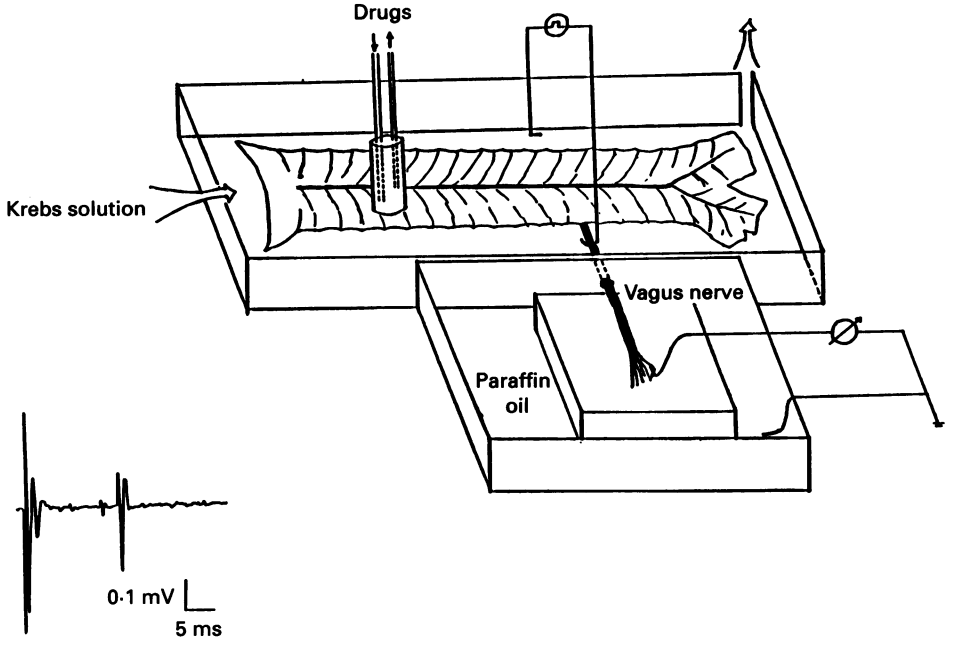

Fig. 1. Schematic diagram of the apparatus used for the recording of single vagal afferent fibres from guinea-pig airways *in vitro*. This comprises a Perspex chamber in which the trachea was pinned (inside surface outwards). The vagus nerve was drawn onto a separate chamber and single filaments split on the surface of a mirror. The vagus nerve trunk was stimulated by a silver hook electrode and single fibre activity was recorded using a monopolar silver electrode. Drugs were superfused onto the surface of the trachea through a plastic cylinder. On the left an example of a single C fibre action potential preceded by the stimulus artifact is illustrated.

Design Ltd, Cambridge, UK). Peristimulus–time histograms and discharge traces were displayed using 'Spike 2·0' CED software. Drug responses were assessed as the number of spikes counted during the 30 s or 1 min application period. All values for responses of single units are given as the mean ± standard error of the mean (S.E.M.), and tests for significance of difference were made with Student's t test or Mann–Whitney U test for two independent samples as appropriate.

Drugs

Stock solutions of all drugs were kept frozen and diluted to their final concentration in Krebs solution on the day of the experiment. Drugs were obtained as follows: capsaicin, bradykinin, [des-Arg^9]-BK and HOE 140 (all synthesized at Sandoz Institute for Medical Research, London), histamine dihydrochloride and 5-HT creatinine sulphate (Sigma, Poole, Dorset), ibuprofen and *m*-chlorophenylbiguanide (Research Biochemicals Incorporated, St Albans, Herts), pentobarbitone sodium (Sanofi Animal Health Ltd, Watford, Middx).

RESULTS

Properties of single fibres

Afferent fibres with conduction velocities below 2·0 m s^{-1} were classified as C fibres and those of less than 25·0 m s^{-1} as Aδ fibres. Aβ fibres were rarely encountered during these experiments, although it is acknowledged that the relatively short distance used for calculating conduction velocities may make it difficult to identify the more rapidly conducting myelinated fibres. Recordings were made from twenty-eight C fibres and twenty-four Aδ fibres and the general properties of these afferents are summarized in Table 1. In general, fibres did not show marked resting activity, although some spontaneous discharge was occasionally evident. Receptive fields were found in the cartilaginous portion of the tissue as well as the trachealis muscle, covering a small area of 1–2 mm diameter, and were evenly distributed from the upper trachea to the right bronchus, although none were found in the left bronchus. There appeared to be no difference in the distribution of Aδ and C fibre receptive fields. Both C and Aδ fibre endings were highly sensitive to mechanical stimuli (Fig. 2) and possessed very low mechanical thresholds, with those for Aδ fibres being significantly lower than those for C fibres ($P < 0{\cdot}01$; Table 1). Responses of both fibre types to a mechanical stimulus usually showed adaptation, and any which showed a slowly adapting response were not included in the present study.

TABLE 1. Summary of the properties of vagal Aδ and C fibres in the guinea-pig trachea *in vitro*

	Aδ	C
Conduction velocity (m s^{-1})	8·4 ± 1·3 (2·7–25·0)	0·9 ± 0·1 (0·4–2·0)
Mechanical threshold (mN)	1·1 ± 0·3 (0·1–4·8)	2·2 ± 0·4 (0·5–6·9)
Chemosensitivity		
Capsaicin	No effect (0·1–3 μM; $n = 12$)	Stimulates (> 30 nM; $n = 20$)
Bradykinin	No effect (3 μM; $n = 7$)	Stimulates (> 0·1 μM; $n = 19$)
Histamine	No effect (10 μM; $n = 8$)	No effect (10 μM; $n = 5$)
5-HT	No effect (10 μM; $n = 5$)	No effect (10 μM; $n = 5$)

In each case n refers to the number of fibres tested with that substance.

Responses to chemicals

Aδ and C fibres showed marked differences in terms of their chemosensitivity (Table 1). Thus, Aδ fibres were insensitive to capsaicin, bradykinin, histamine and 5-HT applied for up to 1 min directly onto their receptive fields. Similarly, histamine, 5-HT and also the 5-HT analogue *m*-chloro-phenylbiguanide (10 μM; $n = 3$) failed to excite C fibres. In contrast, capsaicin and bradykinin evoked a marked firing of all C fibres tested when applied to their receptive fields. Figure 3*A* shows an example of the response of a single C fibre to a 30 s application of capsaicin onto its receptive

field. Typically, there was little delay in the onset of firing after capsaicin application and this activity often continued after the application period. Whether this reflects an on-going activity of the fibre or a lag time in removal of the drug by the perfusion system is not clear. Capsaicin-evoked excitation was seen at a threshold con-

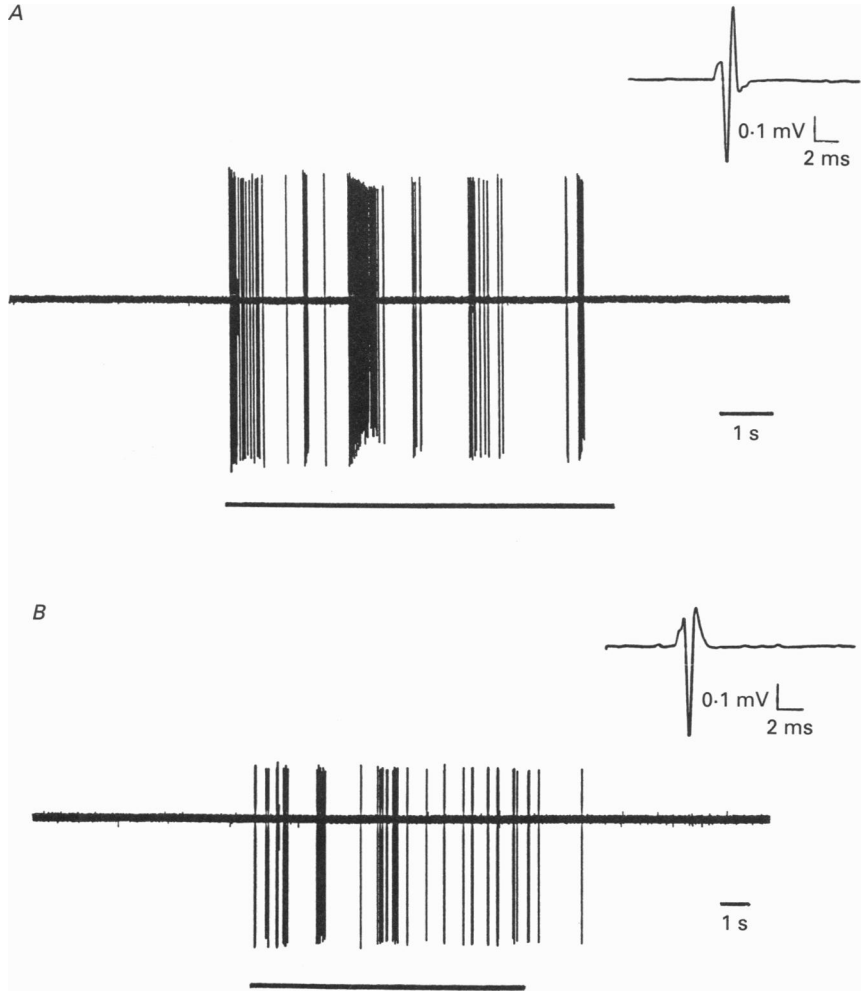

Fig. 2. Responses of a single Aδ fibre (conduction velocity 6·0 m s^{-1}; *A*) and C fibre (conduction velocity 0·84 m s^{-1}; *B*) to mechanical stimulation by gently touching the receptive fields with a blunt glass rod for the periods shown by the bar. Inset are single action potentials evoked by the mechanical stimulus.

centration of 30 nM (mean firing frequency $0{\cdot}9 \pm 0{\cdot}5$ impulses s^{-1}) and a maximal increase in firing was seen at concentrations of 0·1–1 μM (mean firing frequency $9{\cdot}1 \pm 2{\cdot}6$ impulses s^{-1} at 1 μM) indicating a rather steep dose–response relationship (Fig. 3*B*).

Bradykinin produced a pronounced discharge in all C fibres tested. Figure 4*A* shows the response of the same fibre as that in Fig. 3*A* to a 30 s application of bradykinin. Again, there was generally no delay in the response of C fibres to

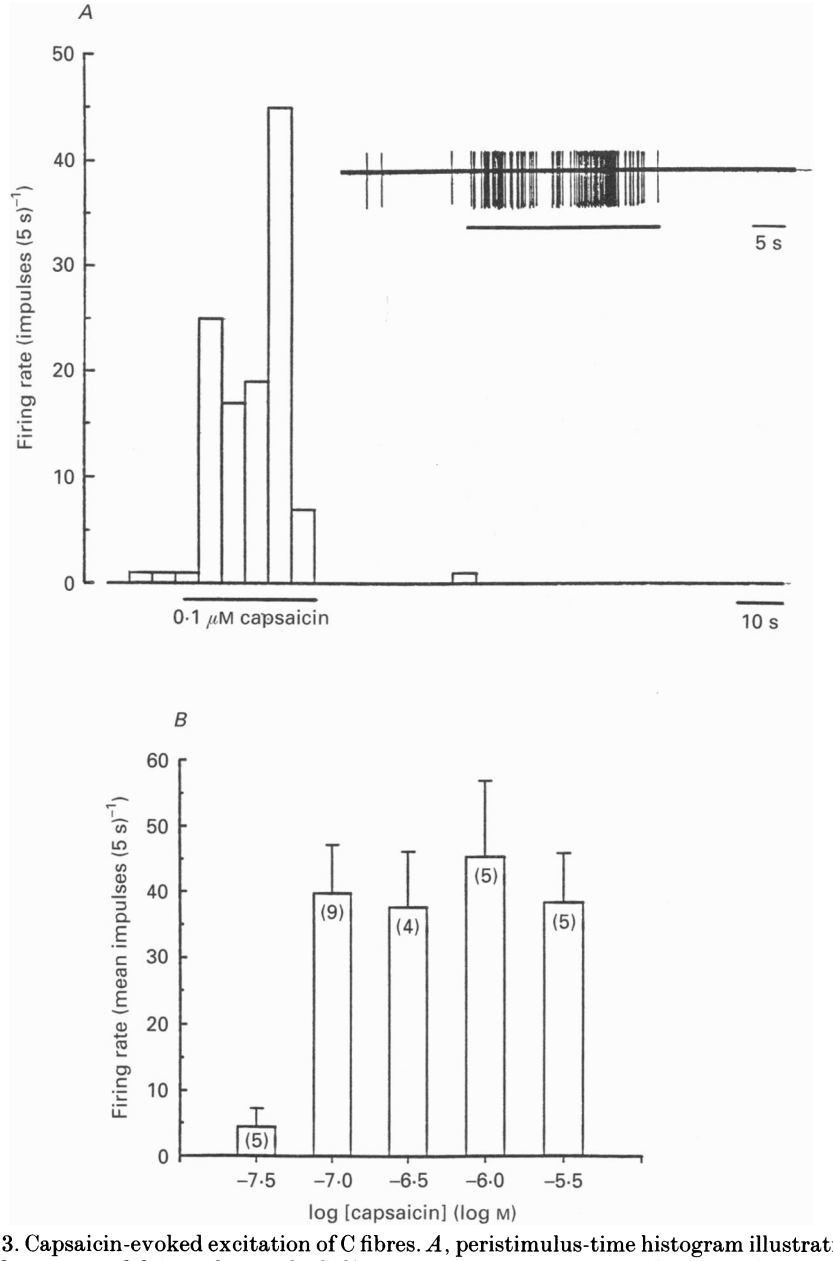

Fig. 3. Capsaicin-evoked excitation of C fibres. *A*, peristimulus-time histogram illustrating the frequency of firing of a single C fibre in response to a 30 s application of capsaicin (0·1 μM) denoted by the bar. The number of impulses counted in 5 s have been plotted. Inset is the recording of the capsaicin-evoked discharge of the fibre. *B*, concentration–response relationship for capsaicin-evoked firing of C fibres. The mean number of spikes per 5 s during a 30 s application period have been plotted. Each column represents the mean ± S.E.M. from the number of fibres indicated in parentheses.

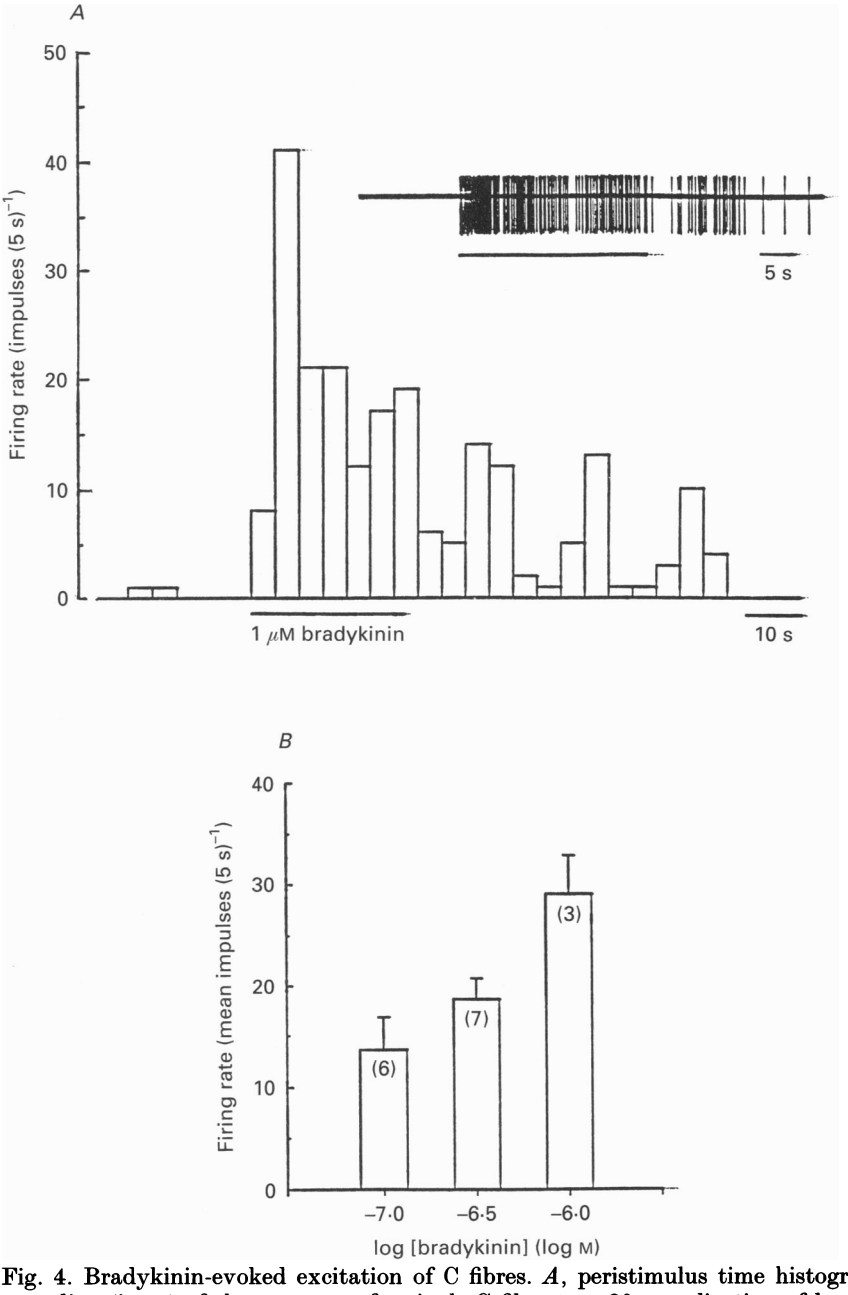

Fig. 4. Bradykinin-evoked excitation of C fibres. *A*, peristimulus time histogram and recording (inset) of the response of a single C fibre to a 30 s application of bradykinin (1 μM) denoted by the bar. Note the on-going activity in this case after removal of the drug. *B*, concentration–response relationship for bradykinin-evoked firing of C fibres. The *Y*-axis represents the mean number of spikes counted per 5 s during a 30 s application period and each column is the mean ± S.E.M. from the number of fibres indicated in parentheses.

bradykinin and on-going activity was also frequently evident. The effect of bradykinin was clearly concentration related (Fig. 4*B*) although it was less potent than capsaicin with a higher threshold concentration of around 100 nM (mean firing frequency $2{\cdot}2 \pm 0{\cdot}6$ impulses s^{-1}) and a peak effect of $5{\cdot}8 \pm 0{\cdot}7$ impulses s^{-1} at 1 μM.

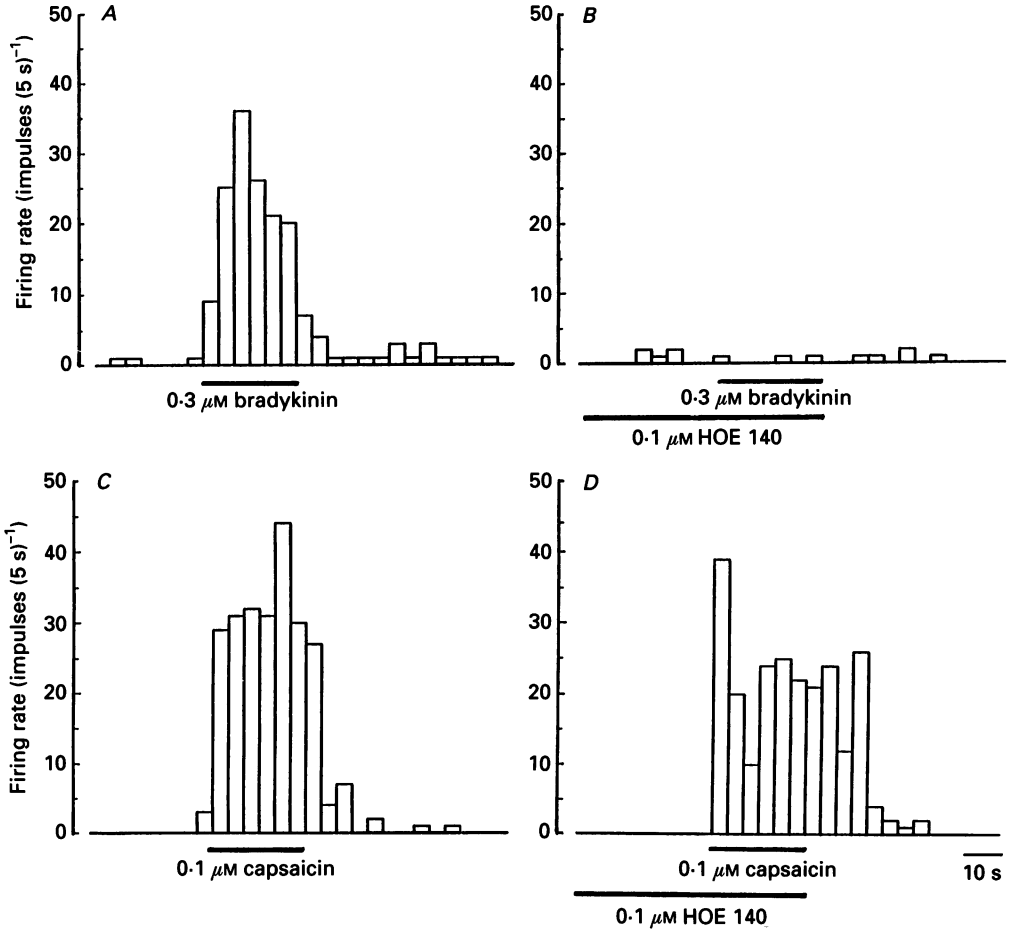

Fig. 5. Antagonism of bradykinin-evoked firing of C fibres by HOE 140. *A*, control response of a single C fibre to a 30 s application of bradykinin (0·3 μM). *B*, after a 5 min perfusion with HOE 140 (0·1 μM) the response to bradykinin, applied 1 h after the control application, was abolished. *C* and *D*, HOE 140 (0·1 μM) was without effect on firing evoked by capsaicin (0·1 μM). Again, 1 h was left between capsaicin applications.

Both capsaicin- and bradykinin-evoked firing did not appear to be influenced by endogenous prostaglandin production since in the presence of ibuprofen, responses to 0·3 μM capsaicin (mean firing frequency $8{\cdot}4 \pm 1{\cdot}0$ impulses s^{-1}; $n = 3$) and 1 μM bradykinin ($6{\cdot}2 \pm 0{\cdot}3$ impulses s^{-1}; $n = 3$) were not significantly different from control values ($7{\cdot}5 \pm 1{\cdot}7$ and $5{\cdot}8 \pm 0{\cdot}7$ impulses s^{-1} respectively; $P > 0{\cdot}05$).

The results obtained with the selective bradykinin receptor subtype ligands indicate that bradykinin-evoked excitation of C fibres was mediated through an interaction with the B_2 receptor subtype. Thus, the selective B_1 receptor agonist

[des-Arg^9]-BK did not produce firing in C fibres at concentrations up to 3 μM ($n = 4$), whilst the selective B_2 receptor antagonist HOE 140 (0·1 μM) abolished responses to a submaximal concentration of bradykinin (0·3 μM; $n = 4$) (Fig. 5*A* and *B*). HOE 140 alone had no effect on the resting state of the fibres. It was not possible to obtain a recovery of the bradykinin response after wash-out of the antagonist, although this was not unexpected since the effect of HOE 140 has been previously reported to be very long lasting (Lembeck, Griesbacher, Eckhardt, Henke, Briephol & Knolle, 1991). However, in separate fibres it was confirmed that reproducible responses to 0·3 μM bradykinin could be obtained in the absence of HOE 140 with no evidence of desensitization (data not shown). Moreover, HOE 140 was without effect against capsaicin-evoked firing (Fig. 5*C* and *D*). Thus, responses to control applications of 0·1 μM capsaicin (mean firing frequency $10{\cdot}6 \pm 1{\cdot}9$ impulses s^{-1}; $n = 3$) were not significantly different when repeated in the presence of 0·1 μM HOE 140 (mean firing frequency $8{\cdot}21 \pm 1{\cdot}7$ impulses s^{-1}; $P > 0{\cdot}05$).

DISCUSSION

The present study provides the first *in vitro* examination of the general properties of single vagal afferents innervating the airways. Recordings were made from Aδ and C fibres which had discrete receptive fields in the trachea and bronchi. To our knowledge, C fibres have not previously been studied using single fibre recording in the guinea-pig, although the conduction velocities seen here are similar to those reported *in vivo* in species such as dogs (Coleridge & Coleridge, 1977), cats (Delpierre, Grimaud, Jammes & Mei, 1981) and rabbits (Karczewski & Widdicombe, 1969) indicating that comparisons may be justified. It is less certain which class of myelinated receptor previously described *in vivo* may correspond to the Aδ fibres seen here. Afferent fibres from rapidly adapting receptors (RARs) conduct in the Aδ range and generally have lower conduction velocities than those from slowly adapting receptors (SARs), although there is some overlap (Sant'Ambrogio, 1987; Karlsson *et al.* 1988). A further indication that Aδ fibres may correspond to RARs comes from their receptive field properties. They were shown here to be distributed throughout the trachea and main bronchi, responding in many cases to the slightest touch of a von Frey hair, with a discharge which was typically rapidly adapting. This would tend to agree with earlier reports that RARs responding to light touch are present in the epithelium throughout the circumference of the trachea and bronchi (Sant'Ambrogio, Remmers, De Groot, Callas & Mortola, 1978). In contrast, extrapulmonary SARs have been localized predominantly to the trachealis smooth muscle (Bartlett, Jeffery, Sant'Ambrogio & Wise, 1976) and respond to airway distension rather than light touch (Widdicombe, 1954).

As previously mentioned (see Introduction), earlier studies on dogs have classified airway C fibre endings as 'bronchial' or 'pulmonary' on the basis of their accessibility to stimulants injected through different routes (Coleridge & Coleridge, 1977). However, a precise localization of these bronchial C fibre endings using mechanical stimuli is not easy in such *in vivo* experiments. With the *in vitro* preparation used here both C and Aδ fibre endings were clearly shown to be distributed along the length of the trachea and right bronchus. Mechanical thresholds for airway sensory receptors have not previously been determined, and in the present

study they were shown to be very low for both C and Aδ fibres. Interestingly, these thresholds were considerably lower than those reported for cutaneous C and Aδ nociceptors (Szolcsanyi, Anton, Reeh & Handwerker, 1988; Seno & Dray, 1993), and also contrasted with those of other visceral afferents. Thus, C fibres with endings in the abdominal viscera, and the majority of afferents supplying the guinea-pig ureter, were found to be sensitive only to noxious mechanical stimuli (Longhurst, Kaufman, Ordway & Musch, 1984; Cervero & Sann, 1989). The extreme mechanical sensitivity of the vagal afferents seen here may well reflect the presumed protective nature of airway receptors and their ability to respond to inhaled irritants (see Karlsson *et al.* 1988).

Examination of the chemosensitivity of Aδ and C fibres showed that Aδ fibres were not stimulated by any of the agents tested. This was perhaps surprising since histamine, 5-HT or its related compound phenylbiguanide, and bradykinin have been reported to stimulate RARs in a number of species including (for histamine) guinea-pig (Mills *et al.* 1969; Dixon *et al.* 1979; Kaufman *et al.* 1980; Bergren & Sampson, 1982). However, there has been some debate as to whether the stimulatory effect of histamine is direct, or indirect as a result of smooth muscle contraction (see also Vidruk, Hahn, Nadel & Sampson, 1977; Yu & Roberts, 1990). The actions of 5-HT and bradykinin on RARs have similarly been suggested to be indirect as a result of bronchial or vascular changes (Dixon *et al.* 1979; Kaufman *et al.* 1980). The lack of effect of all these agents, applied directly onto Aδ fibre receptive fields, in the present study, would tend to support an indirect mechanism of stimulation *in vivo*. Taken together with their extreme mechanical sensitivity, this suggests that these myelinated fibre endings may function primarily as 'irritant' or mechanoreceptors. It should be borne in mind, however, that in cats RARs located in distal airways were thought to be more chemosensitive than those located in the trachea, which had a greater mechanosensitivity (Widdicombe, 1954). It cannot be discounted, therefore, that a subclass of Aδ fibre supplying the smaller airways of the guinea-pig may respond to chemical stimulation.

As with the Aδ fibres, C fibre endings were not stimulated by histamine or 5-HT. Although 5-HT, or the related analogue phenylbiguanide, has long been known to stimulate airway C fibres in a number of species (see Coleridge & Coleridge, 1984), the lack of effect of 5-HT in the present study was not unexpected, as it is only weakly active in depolarizing the isolated vagus nerve of the guinea-pig (Butler *et al.* 1990). This effect is mediated through the 5-HT_3 receptor subtype, and phenylbiguanide is now known to be a selective 5-HT_3 receptor agonist (Ireland & Tyers, 1987). However, this agent together with the more potent analogue *m*-chloro-phenyl-biguanide used here, is inactive in the guinea-pig vagus nerve due to the lower affinity of the 5-HT_3 receptor in guinea-pigs compared to other species (Butler *et al.* 1990). Histamine has also been reported to directly stimulate C fibre endings in dogs (Coleridge & Coleridge, 1977). Its inability to stimulate C fibre endings here may again perhaps be explained by species differences, although this would then be difficult to reconcile with the finding that histamine evokes the release of neuropeptides from capsaicin-sensitive nerves in guinea-pig lung, unless this effect of histamine is indirect (Saria, Martling, Yan, Theodorsson-Norheim, Gamse & Lundberg, 1988).

Capsaicin, widely used as a selective C fibre stimulant, potently stimulated C fibres

when applied, at a range of concentrations, directly onto their receptive fields. This effect of capsaicin, in terms of both threshold and magnitude of response, was greater than that seen in cutaneous C fibres (Foster & Ramage, 1981; Seno & Dray, 1993), again illustrating the apparently greater sensitivity of these airway afferents. It should be noted here that in other species such as rats and cats, capsaicin also excites some cutaneous and visceral Aδ fibres (Longhurst *et al.* 1984; Szolcsanyi *et al.* 1988; Seno & Dray, 1993), and myelinated airway 'irritant' receptors *in vivo* (Mohammed, Higgenbottam & Adcock, 1990). In the present study, capsaicin had no effect on any of the Aδ fibres tested at concentrations up to one hundred times that effective in evoking a discharge in C fibres. These data therefore indicate that in the guinea-pig airways capsaicin may be used as a specific stimulant of C fibres, thereby reinforcing its use in examining the role of these fibres in inflammatory processes (see Introduction).

Bradykinin has previously been demonstrated to excite canine bronchial C fibres *in vivo* (Kaufman *et al.* 1980). The present study demonstrates a direct and potent stimulation of guinea-pig C fibres by bradykinin, and this effect was clearly concentration related. Whilst both C and Aδ fibres of the skin and abdominal viscera appear to be sensitive to bradykinin (Longhurst *et al.* 1984; Lang, Novak, Reeh & Handwerker, 1990), in guinea-pig airways it was selective for C fibres. Furthermore, all C fibres tested were responsive to bradykinin whereas only around 50% of the tested cutaneous fibres were excited. The possible involvement of endogenous prostaglandins in the stimulatory action of bradykinin, and also capsaicin, was investigated since they have been reported to mediate the effects of bradykinin on sensory nerves in some systems (Lembeck, Popper & Juan, 1976), and additionally have a well-established sensitizing action on sensory nerves (Martin, Basbaum, Kwiat, Goetzl & Levine, 1987). However, bradykinin- and capsaicin-evoked discharge of C fibres was present in preparations pretreated with ibuprofen, indicating that it was not dependent on the generation of prostaglandins, or influenced by changes in the reactivity of fibres produced by the possible release of prostaglandins during the dissection process. The influence of a range of other mediators on the reactivity of these C fibres remains to be examined.

The bradykinin-evoked discharge of C fibres was mediated by the B_2 receptor subtype. Thus, the selective B_1 receptor agonist [des-Arg^9]-BK was inactive whilst the B_2 receptor antagonist HOE 140 abolished responses to bradykinin without affecting capsaicin-evoked firing. These results are in keeping with a previous report demonstrating that bradykinin potentiates sensory nerve-mediated contractions in isolated guinea-pig bronchi through a B_2 receptor-mediated mechanism (Miura, Belvisi & Barnes, 1992). The stimulatory effect of bradykinin on C fibres has a pathophysiological significance since in guinea-pigs it causes microvascular leak and, when inhaled, a B_2 receptor-mediated bronchoconstriction. Both effects are partly capsaicin sensitive supporting the involvement of primary afferent fibres (Lundberg & Saria, 1983; Ichinose, Belvisi & Barnes, 1990). An interaction of bradykinin with sensory nerves may well be important in airway inflammation in man since it is present in increased levels in asthmatic patients (Christiansen, Proud, Sarnoff, Juergens, Cochrane & Zuraw, 1992) and inhalation by asthmatics causes a largely reflex bronchoconstriction (Fuller, Dixon, Cuss & Barnes, 1987).

In conclusion, the present study shows that the properties of airway afferents may be studied directly *in vitro* and provides the first examination of these fibres in the guinea-pig. Whilst it is difficult with this preparation to study pulmonary sensory receptors, its use does enable the direct examination of airway sensory fibres without the problems and ambiguities inherent with whole-animal studies. Further experiments may provide an insight into the interaction of these fibres with a variety of mediators and their role in inflammatory processes.

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