Effects of noradrenaline on rat paratracheal neurones and localization of an endogenous source of noradrenaline

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1 Intracellular recording techniques were used to study the actions of exogenous noradrenaline (NA) on rat paratracheal neurones *in situ*. The receptor subtypes underlying these actions were investigated by application of selective adrenoceptor antagonists.

2 Application of NA (0.1–10 μM) by superfusion evoked a membrane depolarization in 85% (52 out of 61) of all paratracheal neurones studied. The response consisted of a slow depolarization which was sometimes accompanied by action potential discharge. In 26 out of 31 cells the response was associated with a change in input resistance of the cell membrane. In 22 out of 26 cells there was a 30% increase, whilst in a further 4 cells there was a 15% decrease in input resistance. The amplitude of the NA depolarization was concentration-dependent.

3 The depolarization evoked by NA was reversibly antagonized by prazosin (1 μM) but unaffected by yohimbine (1 μM) or propranolol (1–10 μM).

4 High performance liquid chromatography with electrochemical detection (h.p.l.c.-e.c.d.) was used to assay for NA and dopamine in samples containing mainly paratracheal ganglia and in samples of tracheal smooth muscle with mucosa. NA was present in all samples assayed at a level of 1.6 $\mu\text{g NA g}^{-1}$ and 0.5 $\mu\text{g NA g}^{-1}$ wet weight of the two sample types respectively. Dopamine was not detected in any samples of either ganglia or smooth muscle with mucosa.

5 It is concluded that NA-evoked depolarizations of rat paratracheal neurones result from stimulation of α_1 -adrenoceptors, and that local levels of NA may be sufficiently high to activate these receptors directly.

Keywords: Trachea; ganglia; noradrenaline

Introduction

The intramural parasympathetic ganglia of the airways are thought to be the integrative sites of the neural output controlling smooth muscle tension, mucosal secretion and tracheobronchial reflexes (Richardson, 1979). The parasympathetic intracardiac (Jacobowitz, 1967) and vesical ganglia (Hamberger & Norberg, 1965a,b; Norberg & Sjöqvist, 1966; De Groat & Saum, 1971) have been shown to be adrenergically innervated.

Three membrane responses to noradrenaline (NA) have been recorded in various autonomic ganglia: hyperpolarizations, depolarizations; and biphasic responses consisting of a hyperpolarization followed by depolarization (Morita & North, 1981; Nakamura *et al.*, 1984; North & Surprenant, 1985; Akasu *et al.*, 1985). In the parasympathetic submandibular and vesical ganglia, the most frequently observed response to NA is a hyperpolarization mediated through α_2 -adrenoceptors (Suzuki & Volle, 1979; Akasu *et al.*, 1985). Similarly, in sympathetic superior cervical ganglia, hyperpolarization to NA is mediated by α_2 -adrenoceptors (Ashe & Libet, 1982). In contrast, the depolarizations in parasympathetic ganglia have been shown to be mediated through α_1 -adrenoceptors (Akasu *et al.*, 1985) and in sympathetic ganglia they are mediated through β -adrenoceptors (De Groat & Volle, 1966; Brown & Dunn, 1983). Noradrenaline has also been shown to inhibit excitatory nicotinic neurotransmission in the paratracheal ganglia of the ferret (Baker *et al.*, 1983) and in parasympathetic ganglia of urinary bladder (Tsurasaki *et al.*, 1990).

Evidence for adrenergic innervation of paratracheal ganglia is sparse. However, adrenergic nerve fibres have been found terminating near airway ganglia of the calf (Jacobowitz *et al.*, 1973; Phipps *et al.*, 1982) and noradrenergic varicose fibres have also been localized in the mucosa, tracheal muscle and around arteries in the rat trachea (Baluk & Gabella, 1989). Whilst adrenergic fibres were found to be absent from rat paratracheal ganglia, a population of the neurones have been shown to express partially a noradrenergic phenotype (Baluk & Gabella, 1989).

In this paper we describe the effect of NA on rat paratracheal neurones and attempt to determine the receptor subtypes responsible for mediating this response. In addition, we have carried out assays for NA and dopamine in order to determine local catecholamine levels. Finally, we discuss the possible implications of these findings for airway function.

Methods

All experiments were performed on 15–18 day old Sprague-Dawley rat pups of either sex. Animals were stunned by a blow to the back of the head and then killed by cervical dislocation. The trachea was excised from the base of the carina to the bifurcation of the main bronchi and cut midline along the length of its ventral surface. The trachea was pinned out, dorsal side uppermost, onto a small block of Sylgard (Dow Corning) prebonded to a glass slide which formed the base of the recording chamber. The adventitia were then removed to expose the intramural paratracheal ganglia. The assembled chamber was then clamped to the stage of a Zeiss Ergoval microscope equipped with Hoffman

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modulation contrast optics giving 40 and 640 fold magnification. The preparation was superfused at a rate of 6 ml min^{-1} with Krebs solution of composition (mM): NaCl 118.1, KCl 4.7, MgSO_4 1.2, KH_2PO_4 1.2, CaCl_2 2.5, NaHCO_3 25, glucose 10, and gassed with 95% O_2 /5% CO_2 . The temperature was maintained at $34\text{--}36^\circ\text{C}$ by a remote thermostatically-controlled heating coil and monitored by a thermistor positioned next to the trachea. Impalements were made with electrodes having d.c. resistances between 80 and $110 \text{ M}\Omega$ and filled with 2.3 M potassium citrate (pH 7.1). The electrodes were connected to an amplifier with an active bridge circuit that allowed simultaneous current injection and voltage recording (Axoclamp 2A). Electrode resistance and tip offset potentials were nulled before impalement to allow estimations of input resistance and membrane potential to be made during the recording. In order to aid placement of electrodes and impalement of neurones, the final 2–3 mm of the electrodes were bent through an angle of $45\text{--}70^\circ$ before being filled. Input resistance measurements were made by passing 100 ms current pulses of known intensity across the cell membrane and measuring the amplitudes of the hyperpolarizations evoked. Drugs were applied by bath superfusion from small drug reservoirs connected by a multi-way tap. The time for drugs to reach the bath from the reservoirs was approximated by use of fast green dye. This was found to be around 40 s. Data were either stored on tape for future analysis (Racal store 4DS) or displayed on a Textronix storage oscilloscope (Model D13) and a Gould pressure ink recorder (Model 2200 S).

H.p.l.c. analysis of paratracheal ganglia and smooth muscle with mucosa

Tracheae from 15–17 day old rats were dissected and pinned out as previously described. The paratracheal ganglia were then dissected from the trachealis under $\times 50$ magnification and stored in liquid nitrogen. The underlying smooth muscle with mucosa was then dissected and also stored in liquid nitrogen. Samples were pooled from 5–6 animals and high performance liquid chromatography with electrochemical detection (h.p.l.c.-e.c.d.) was used to measure the levels of NA and dopamine present. The pooled samples of dissected ganglia and muscle were blotted dry, weighed and homogenized in $500 \mu\text{l}$ 0.1 M perchloric acid/ 0.4 mM sodium bisulphite containing 25 ng ml^{-1} dihydroxybenzylamine (DHBA) as an internal standard. After centrifugation the supernatants were subjected to alumina extraction as described

previously (Griffith *et al.*, 1982). NA and DHBA were eluted from the alumina into $300 \mu\text{l}$ 0.1 M perchloric acid/ 0.4 mM sodium bisulphite and $50 \mu\text{l}$ samples were injected for h.p.l.c.-e.c.d. Separation was carried out on a $10 \text{ cm } 5 \mu\text{m}$ C18 reverse phase column (Spherisorb 500DS2, Hichrom) with a mobile phase of 0.1 M phosphate buffer (pH 5.0), 0.1 mM ethylenediaminetetraacetate (EDTA) and 5 mM heptane sulphate containing 10% (v/v) methanol (Moyer & Jiang, 1978) at a flow rate of 2 ml min^{-1} . Quantification was achieved with a Coulochem detector (Model 5100A, Severn Analytical) set at the following potentials: guard cell, $+0.25 \text{ V}$; electrode 1, $+0.21 \text{ V}$; electrode 2, -0.23 V . Concentrations of NA and DHBA were determined by reference to the elution times and peak heights of standard solutions and the concentration of NA was corrected for recovery from the alumina extraction by use of the DHBA internal standard.

Statistical analysis

Results are expressed as means $\pm$ s.e.mean. Differences between means were analysed by Student's *t* test (paired or unpaired as appropriate). A *P* value of less than or equal to 0.05 was considered to be significant.

Drugs

Noradrenaline bitartrate, prazosin hydrochloride and yohimbine hydrochloride were obtained from Sigma, United Kingdom. Propranolol hydrochloride was obtained from ICI, United Kingdom. Noradrenaline and propranolol were dissolved in $100 \mu\text{M}$ ascorbic acid at a concentration of $100 \mu\text{M}$. Yohimbine was dissolved in 25% methanol at a concentration of 1 mM . All drugs were diluted in Krebs solution for superfusion.

Results

Membrane responses to noradrenaline

Superfusion of NA ($0.1\text{--}10 \mu\text{M}$) onto rat paratracheal neurones evoked a slow depolarization in 85% (52 out of 61) of neurones. Of 49 neurones (RMP = $48.6 \pm 1.0 \text{ mV}$) tested with $10 \mu\text{M}$ NA, 46 (94%) were depolarized ($8.3 \pm 0.7 \text{ mV}$) (Figure 1). In 55% of these neurones, this depolarization was accompanied by action potential discharge (Figure 1). The amplitude of the evoked depolarization was concentration-

Figure 1 The effects of superfusion of $10 \mu\text{M}$ noradrenaline (NA) on rat paratracheal neurones. The open channel of the multiway tap is connected to the drug reservoir at the point indicated by $\blacktriangle$, and reconnected to the Krebs solution at the point indicated by $\blacktriangledown$. An artefact is occasionally seen at these points. (a) NA elicited a slow depolarization which was accompanied by an increase in input resistance of the membrane (from $10 \text{ M}\Omega$ resting to $14 \text{ M}\Omega$). Resting membrane potential -62 mV . (b) In another cell, the NA-evoked depolarization was accompanied by spontaneous firing of action potentials. Resting membrane potential -50 mV , input resistance $9 \text{ M}\Omega$. The downward deflections are the membrane responses to constant current hyperpolarizing pulses (100 pA), as an indicator of membrane input resistance changes.

dependent with 0.1, 1 and 10 μM NA producing a depolarization of 0.8 ± 0.8 mV ($n = 6$), 4.1 ± 1.5 mV ($n = 6$) and 8.3 ± 0.7 mV ($n = 49$) respectively. A change of input resistance of the cell membrane accompanying the response to NA was not easily detected. Of 26 cells in which an apparent change in input resistance accompanying the NA-evoked depolarizations was observed, 22 cells displayed an increase in resting resistance ($30 \pm 5\%$) and 4 a small decrease ($15 \pm 6\%$). In one cell there was no change in resistance accompanying the rising phase of the depolarization although the falling phase was accompanied by a decrease in input resistance. In a further 4 neurones no change of input resistance was observed. The depolarization was unaffected by 0.5 μM tetrodotoxin (TTX) indicating no involvement of intermediary synapses.

Receptors mediating the noradrenaline response

The effects of adrenoceptor antagonists were examined to determine the receptor subtype responsible for mediating the NA response (Table 1, Figure 2). Prazosin (1 μM), a selective α_1 -adrenoceptor antagonist abolished the depolarization elicited by 10 μM NA in all neurones tested ($n = 8$). Prazosin was difficult to wash out but the reversibility of the antagonism was shown in 4 cells. Prazosin (0.1 μM) exhibited

less effective antagonism of the response to NA showing 40% inhibition ($n = 2$). High concentrations of NA (4 mM) were required to overcome the inhibition by 1 μM prazosin ($n = 2$). Yohimbine (1 μM), a selective α_2 -adrenoceptor antagonist had no significant effect on the NA depolarization ($n = 8$). Propranolol (10 μM), a non selective β -adrenoceptor antagonist, did not inhibit the depolarization evoked by 10 μM NA ($n = 4$). In a further 4 cells the NA response also persisted in Krebs solution containing 10 μM propranolol. Phenylephrine (10 μM), an α_1 -adrenoceptor agonist also elicited a depolarization similar to that produced by NA (unpublished observation).

H.p.l.c. analysis of catecholamine levels in paratracheal ganglia and of smooth muscle with mucosa

The samples containing paratracheal ganglia and those containing smooth muscle with mucosa were analysed by h.p.l.c. techniques for NA and dopamine. NA was found in both paratracheal ganglia samples (1.6 ± 0.4 μg NA g^{-1} tissue w.wt.) and smooth muscle with mucosa samples (0.5 ± 0.1 μg NA g^{-1} tissue w.wt.). These values were significantly different ($P < 0.05$, $n = 6$). Dopamine was not detected in any samples of either paratracheal ganglia or smooth muscle with mucosa.

Figure 2 The effects of the α -adrenoceptor antagonists, prazosin (1 μM) and yohimbine (1 μM) and the β -adrenoceptor antagonist, propranolol (10 μM) on the response to 10 μM noradrenaline (NA). The paratracheal neurones were superfused with antagonist for 2 min before superfusion with both antagonist and NA. The open channel of the multiway tap was connected to the reservoir containing NA or NA and antagonist at the point indicated by $\blacktriangle$ and reconnected to Krebs solution at the point indicated by $\blacktriangledown$. An artefact is occasionally seen at these points. (a) The effect of NA in the absence (upper panel) and presence (lower panel) of 1 μM prazosin. In the presence of prazosin both the depolarization and the spontaneous firing evoked by NA were abolished. Resting membrane potential -45 mV, input resistance 57 $\text{M}\Omega$. (b) The effect of NA in the absence (upper panel) and presence (lower panel) of 1 μM yohimbine. In the presence of yohimbine the onset of depolarization was delayed. This was probably the consequence of an increased time taken for the NA and yohimbine to reach the bath from the reservoir. Resting membrane potential -60 mV, input resistance 143 $\text{M}\Omega$. (c) The effect of NA in the absence (upper panel) and presence (lower panel) of 10 μM propranolol. Resting membrane potential -50 mV, input resistance 88 $\text{M}\Omega$. The downward deflections are the membrane voltage responses to constant hyperpolarizing current pulses (50 pA), as an indicator of membrane input resistance changes.

Table 1 Effects of adrenoceptor antagonists on the response to 10 μ M noradrenaline (NA)

	(μ M)	Amplitude of depolarization (mV)		Resting membrane potential (mV)	(n)
		Control (NA)	Drug		
Prazosin	(1)	7.9 $\pm$ 0.4	0***	51.5 $\pm$ 0.9	8
Yohimbine	(1)	7.5 $\pm$ 0.9	6.3 $\pm$ 0.7 NS	57.1 $\pm$ 3.9	8
Propranolol	(10)	6.8 $\pm$ 0.7	6.8 $\pm$ 0.7 NS	45.9 $\pm$ 1.5	8

***Significant difference $P < 0.001$ between responses in the absence and presence of antagonist. NS = no significant difference.

Discussion

The primary aims of these experiments were to determine the response of rat paratracheal neurones to exogenous NA and to detect any NA present in the ganglia.

The findings of the present study showed that NA exerted a powerful and direct effect upon a high proportion (85%) of the parasympathetic intramural neurones of rat trachea. In over half of these neurones the depolarization produced by NA raised the excitability of the cell sufficiently to evoke firing of action potentials. The apparent increase in the input resistance of the cell membrane which accompanied the depolarization in some of the cells suggests a decrease in conductance of the membrane. A similar response has previously been observed in vesical parasympathetic neurones where the depolarization evoked by NA was also mediated via α_1 -adrenoceptors (Akasu *et al.*, 1985). In other autonomic ganglia NA has been shown to evoke hyperpolarizations, depolarizations and biphasic responses. Furthermore, in vesical parasympathetic ganglia and enteric ganglia the most frequently observed response to NA is a hyperpolarization (Hirst & Silinsky, 1975; Akasu *et al.*, 1985; North & Surprenant, 1985). In contrast to other parasympathetic ganglia, the rat paratracheal neurones in the present study were consistently depolarized by NA and no paratracheal neurones studied were hyperpolarized by NA.

It has been shown that in both myenteric neurones and parasympathetic ganglia, the hyperpolarization elicited by NA is mediated by α_2 -adrenoceptors and the depolarizations are mediated by α_1 -adrenoceptors (Morita & North, 1981; Akasu *et al.*, 1985; Schemann, 1991). This is in contrast to sympathetic ganglia where the NA-induced hyperpolarization has been shown to be mediated by α_2 -adrenoceptors and the depolarization by β -adrenoceptors (Brown & Dunn, 1983). In the present experiments it was found that the depolarization evoked by NA was reversibly inhibited by prazosin and unaffected by yohimbine and propranolol. Furthermore the depolarization elicited by NA was mimicked by the α_1 -adrenoceptor agonist, phenylephrine.

In conclusion, the depolarization of rat paratracheal neurones by NA appears to be mediated by α_1 -adrenoceptors. This is consistent with other parasympathetic ganglia where a depolarization to NA is also mediated by α_1 -adrenoceptors. It has been determined that rat paratracheal neurones exhibit two extremes of behaviour in response to prolonged intrasomal current injection (Allen & Burnstock, 1990a). However, with respect to their response to NA, they appear to be a homogeneous population, as they were reported to be in response to γ -aminobutyric acid (GABA) (Allen & Burnstock, 1990b).

Given the powerful effect that NA exerted upon rat paratracheal neurones it is important to determine if there is any endogenous NA within the airways. Baluk & Gabella (1989) have shown that although the trachea is sympathetically innervated, the paratracheal ganglia themselves lack a sympathetic innervation. However, they have also demonstrated that the paratracheal neurones express a partial noradrener-

gic phenotype and are capable of taking up amine precursors and under some circumstances may synthesize NA (Baluk & Gabella, 1989). Nonetheless, the release of NA from these neurones has not yet been reported. If there are no sympathetic nerve pericellular baskets around the ganglia, it is pertinent to ask how NA reaches paratracheal neurones. Other possible sources of NA to be considered which could act upon these neurones are from small intensely fluorescent (SIF) cells and circulating NA. SIF cells have been found to contain catecholamines and peptides (Elfvin *et al.*, 1975; Pakpa *et al.*, 1987) and in the autonomic nervous system SIF cells have been shown to perform both interneuronal and paraneuronal roles (Williams & Jew, 1983). The interneuronal role has been observed in the superior cervical ganglion where concomitant changes in formaldehyde-induced fluorescence of the SIF cells and in slow inhibitory postsynaptic potentials associated with release of catecholamines were induced by stimulation of the preganglionic nerve (Libet & Owman, 1974). Furthermore, they have been located histochemically in the rat trachea within the paratracheal ganglia and associated with blood vessels (Baluk & Gabella, 1989). In the rat, NA is a presumptive SIF cell transmitter (Eränkő & Eränkő, 1971; Lever *et al.*, 1976; König & Heym, 1978). It is possible that under some conditions the contents of the SIF cells could be released and act locally on the paratracheal neurones or be released into the blood vessels with which they are associated and transported in the blood to act on neurones at some distance from the point of release (paracrine role). Intraganglionic blood vessels have been shown to be present in paratracheal ganglia (Baluk *et al.*, 1985). In the present study the samples containing ganglia were assayed for, and found to contain, NA. There was a significantly greater level of NA in the samples of paratracheal ganglia compared with those of smooth muscle with mucosa ($P < 0.05$). If the NA detected is from SIF cells located within the ganglia, it is quite plausible that locally released NA can be of sufficient levels to depolarize the neurones and thereby modulate their excitability. This could have consequences for airway function. Previously, it has been shown that rat paratracheal neurones display a high level of subthreshold spontaneous synaptic activity (Allen & Burnstock, 1990a). High levels of subthreshold synaptic activity have also been observed in cat tracheal ganglia *in vivo*, and it has been suggested that synchronization of multiple inputs are required to elicit action potential discharge in these cells (Mitchell *et al.*, 1987). The decrease in membrane potential elicited by NA in this study would mean that the overall intensity of vagal and/or laryngeal nerve input required to elicit action potential discharge would be decreased by NA acting on α_1 -adrenoceptors and raising the excitability of the ganglion cells.

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