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Histochemical localization of adrenergic nerves in the guinea-pig trachea

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Summary

1. Specific catecholamine fluorescence was demonstrated in guinea-pig trachea in fine, varicose, nerve fibres running parallel to the tracheal smooth muscle fibres.

2. The density of nerves in tracheal smooth muscle was greater at the laryngeal end than at the bronchial end of the trachea.

3. The findings confirm pharmacological evidence for an adrenergic innervation of the guinea-pig isolated tracheal chain preparation.

Introduction

The isolated tracheal chain preparation of the guinea-pig is frequently used in quantitative pharmacological studies on the potency of sympathomimetic amines with β-adrenoceptor activity. These studies involve a comparison of dose-response lines, and factors which might influence the position of these lines should be taken into account e.g. uptake into adrenergic nerves (Chahl & O'Donnell, 1967). Because the tracheobronchial tree is considered to be supplied by sympathetic nerves which are dilator (Widdicombe, 1966) it has been assumed that the guinea-pig trachea is adrenergically innervated. This assumption has been supported by indirect pharmacological evidence, e.g. relaxation of isolated tracheal preparations produced by transmural electrical stimulation (Foster, 1964) or indirectly acting sympathomimetic amines (Chahl & O'Donnell, 1971), and potentiation of responses of some amines when neuronal uptake is inhibited by cocaine (Chahl & O'Donnell, 1967; Foster, 1967). However, in a brief reference to guinea-pig trachea, Hollands & Vanov (1965) described it as a sparsely innervated tissue with adrenergic nerves being confined to blood vessels. The present study aimed to examine the adrenergic innervation of the guinea-pig trachea more closely.

Methods

Adrenergic nerves were visualized in sections of trachea by the Falck-Hillarp fluorescent histochemical technique (Falck, 1962). Tracheae were removed from female guinea-pigs (350–550 g) and cut into rings. In some experiments these rings were quenched immediately in isopentane, cooled to $-130°$ C in liquid nitrogen. In other experiments they were washed for 30 min in Krebs solution before quenching. The tissues were then freeze-dried for 72–96 h at $-45°$ C and at least 10^{-3} Torr. After returning the tissues to room temperature, they were transferred quickly to a desiccator containing paraformaldehyde which had been stored for at least 4 days at a relative humidity of 70%. The desiccator was sealed

and placed for 1 h in an oven at 80° C. The tissues were then infiltrated with 53–55° C Paraffin Embedding Compound (Will Scientific Inc., U.S.A.) at 62° C for 3–5 h, embedded in 53–55° Paraffin Embedding Compound and sectioned at 10 μm. Sections were dry mounted in liquid paraffin and viewed for fluorescence with a Leitz fluorescence microscope, using a 3 mm BG 12 excitation filter, a 530 nm barrier filter placed above the microscope objectives and a dark field condenser. Photographs were taken with a Leitz Automatic camera on Kodak Tri-X film.

In some animals the adrenergic nerves were depleted of catecholamines by pretreatment of the animals with 5 mg/kg reserpine intraperitoneally 24 h previously. In other animals the fluorescence brightness of the adrenergic nerves was enhanced by pretreating the animal with 100 mg/kg nialamide intraperitoneally 2 h before the experiment and then incubating the tracheal rings in 5×10^{-6}M noradrenaline for 15 min before quenching.

To assist in identification of structures, the light microscope was used to examine (a) the general histology of the trachea after staining with haematoxylin and eosin or (b) the distribution of elastic fibres after staining with Verhoeff & Van Gieson's stain.

Drugs used were: (−)-noradrenaline bitartrate (Sigma), nialamide (Pfizer and Co.) and reserpine (Serpasil, Ciba).

Results

After 1 h exposure to formaldehyde vapour, green–yellow fluorescence was observed in the tracheal smooth muscle, in blood vessels and in close proximity to glands. This fluorescence was characteristic of primary catecholamines since no further fluorescence was developed after prolonging the exposure time to 3 hours. Fluorescence was not seen if exposure to formaldehyde vapour was omitted or if the tissues were taken from animals which had been pretreated with reserpine (Fig. 1D). Treatment of animals with a monoamine oxidase inhibitor and incubation of the trachea in noradrenaline caused the specific fluorescence to be greatly enhanced and resulted in the fluorescent fibres appearing less beaded (Fig. 1C).

Most smooth muscle fibres in the guinea-pig trachea run transversely in the dorsal wall of the trachea and bridge the two ends of the U-shaped cartilage. A transverse section through the trachea showed these smooth muscle bands in longitudinal view and fine fluorescent nerve fibres were seen running parallel to the muscle fibres (Figs. 1A and 1B). Under higher magnification (Fig. 2) the innervation of the tracheal smooth muscle was seen as a ramifying network of varicose fibres characteristic of an adrenergic ground plexus (Malmfors, 1965). In addition, longitudinal and oblique bundles of smooth muscle fibres were occasionally observed, particularly in the bronchial end of the trachea. In a cross section through the trachea the adrenergic nerves accompanying these bundles were seen as bright fluorescent points.

The density of innervation seen in the tracheal smooth muscle varied considerably from animal to animal; it also varied with the level of the trachea at which the section was made. In particular, it was noticeable that the ring closest to the larynx was always more densely innervated than that closest to the bronchi (compare Fig. 1A and 1B).

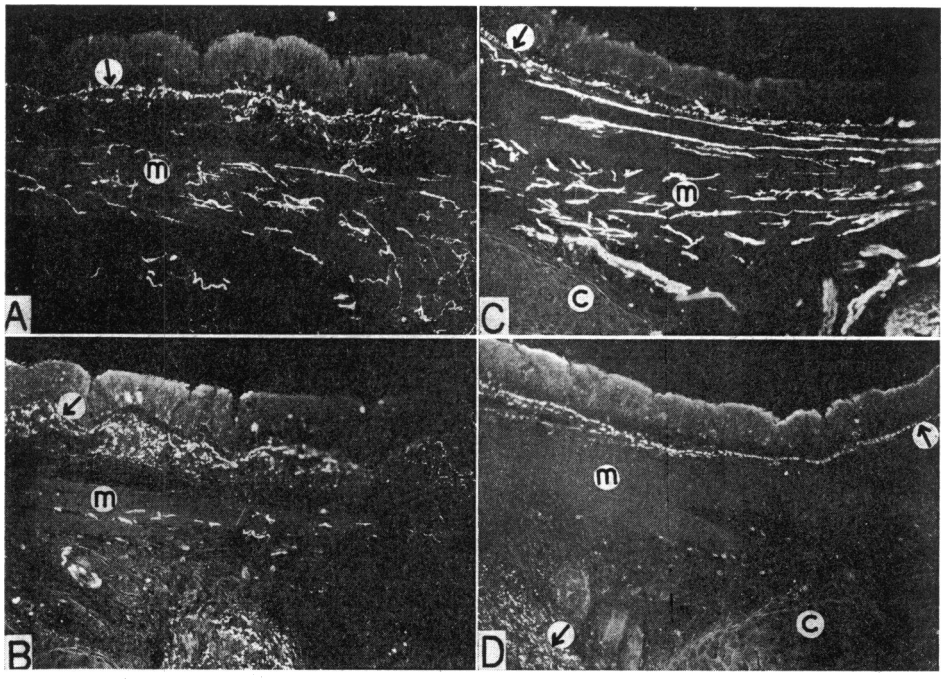

FIG. 1. Transverse sections of guinea-pig trachea (×125) (A) through the laryngeal end and (B) through the bronchial end of a trachea from an untreated animal. In (C) the section is through the laryngeal end of a trachea from an animal pretreated with nialamide. The ring was incubated for 15 min in 5×10^{-6}M noradrenaline. In (D) the section is through a ring taken from an animal pretreated with reserpine. m is the tracheal smooth muscle, c is the cartilage. Autofluorescent elastic fibres are arrowed.

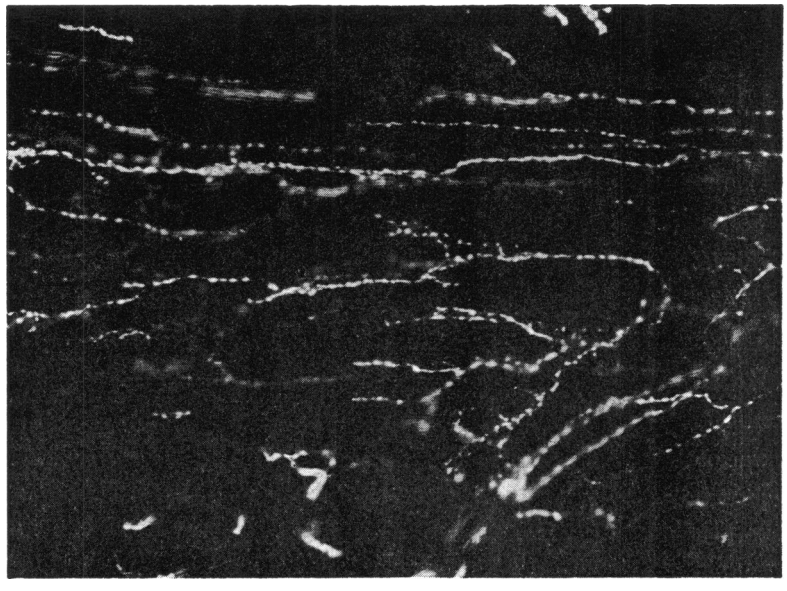

FIG. 2. Transverse section through the guinea-pig trachea (×312·5). This illustrates the plexus of fine, fluorescent, varicose fibres innervating the tracheal smooth muscle.

Background fluorescence of the trachea was slight but bright autofluorescence, confirmed as due to elastic fibres (Leeson & Leeson, 1970), was observed in the lamina propria (Fig. 1), in the submucosa near glands, in the adventitia (Fig. 1D), and in the intima of arteries. This was also seen in tracheae from reserpine-treated animals (Fig. 1D) and in tracheae which had not been exposed to formaldehyde.

Discussion

Fluorescent nerves were seen in the guinea-pig tracheal smooth muscle, appearing as fine, beaded, anastomosing fibres orientated parallel to the smooth muscle cells. Their overall density was not great in comparison with that seen in other tissues from this species e.g. atria, vas deferens. Nevertheless, the present results support the pharmacological evidence that guinea-pig tracheal smooth muscle is adrenergically innervated. There was, however, a noticeable variation in the innervation of the tracheal smooth muscle between different ends of the trachea. Nerves were usually clearly visible in the muscle of the laryngeal ring whereas innervation was sparse at the bronchial end. Hollands & Vanov (1965) may have failed to see innervation to the tracheal smooth muscle because they used sections of trachea taken from nearer the bronchial end of the trachea. Foster & O'Donnell (1972) found that the firmly-bound uptake of ^{3}H-(−)-noradrenaline, which they assumed was into adrenergic nerves, was greater in the laryngeal half of the trachea than in the bronchial half. Foster & O'Donnell (unpublished observations) also found the catecholamine content to be 4·83 nmol/g in the laryngeal half and only 1·19 nmol/g in the bronchial half of the trachea. A functional significance of this difference in nerve density is not clear at present.

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