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A METHOD FOR RECORDING SMOOTH MUSCLE AND VASCULAR RESPONSES OF THE BLOOD-PERFUSED DOG TRACHEA *In situ*

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1 A method is described for measuring responses of dog tracheal musculature and vasculature *in situ*.

2 The upper two thirds of the trachea was perfused with blood through both cranial thyroid arteries at a constant pressure. The blood flow through the arteries was measured with an electromagnetic flowmeter. The response of the tracheal musculature was measured as a change in pressure in a water-filled cuff inserted into the trachea *via* the mouth. Drugs were injected close-arterially.

3 Acetylcholine produced dose-dependent increases in blood flow rate (vasodilatation) and in tracheal intraluminal pressure (tracheal constriction). These responses were antagonized by atropine.

4 Isoprenaline produced vasodilatation which was blocked by propranolol. Adrenaline and noradrenaline caused vasoconstriction which was blocked by phentolamine.

5 All three catecholamines produced a decrease in tracheal intraluminal pressure (tracheal dilatation). The tracheal dilatation in response to adrenaline and noradrenaline was converted to constriction by propranolol. The tracheal constriction thus unmasked was abolished specifically by phentolamine.

6 From these results it is concluded that the tracheal musculature and vasculature contain muscarinic receptors, and excitatory α- and inhibitory β-adrenoceptors. In the tracheal musculature β-adrenoceptors predominate over α-adrenoceptors; the reverse is true in the tracheal vasculature.

Introduction

In the past decade many studies have been made of the physiological and pharmacological properties of the tracheal smooth muscle. Mammalian species used in these studies include the calf (Ariëns, 1967), the cat (Akçasu, 1959; Maengwyn-Davies, 1968; Fleisch, Maling & Brodie, 1970), the guinea-pig (Castillo & De Beer, 1947; Akçasu, 1959; Jamieson, 1962; Foster, 1966; Takagi, Osada, Takayanagi & Taga, 1967; Danko, Tozzi & Roth, 1968; Kasses, Beinfield, Seifter & Seifter, 1968; Chahl & O'Donnell, 1969; Everitt & Cairncross, 1969; Guirgis, 1969; Lynn James, 1969; Fleisch *et al.*, 1970; Rikimaru & Sudoh, 1971; Chahl, 1972; Coleman & Levy, 1974; Kirkpatrick, 1975), the rabbit (Akçasu, 1959; Fleisch *et al.*, 1970) and the rat (Akçasu, 1959; Fleisch *et al.*, 1970). However, most of the studies have been on isolated tracheal preparations bathed in physiological salt solutions. Only one study (Lynn James, 1969) used the *in vivo* tracheal preparation of the guinea-pig to assess drug action on the tracheal musculature. In view of this, it was felt that *in situ* recording of tone of the tracheal musculature with its nerve and blood supply intact would be of value and might reveal some features of the tracheal musculature which have previously escaped notice in *in vitro* experiments. Thus, in the present experiments we attempted to perfuse the tracheal vasculature with blood through the arteries and to record tracheal muscle tone in the dog. This method enabled us to inject drugs selectively into the tracheal vasculature and to observe simultaneously vascular and muscular responses of the trachea. The use of the method is illustrated with experiments on the interactions between cholinoceptor agonists and antagonists and adrenoceptor agonists and antagonists.

Part of this work was briefly reported at the 48th general meeting of the pharmacological society of Japan (Himori & Taira, 1975).

Methods

Experiments were performed on 38 adult mongrel dogs of either sex, weighing 12.5–18.0 kg. Anaesthesia was induced by a single intravenous injection of pentobarbitone sodium 30 mg/kg and

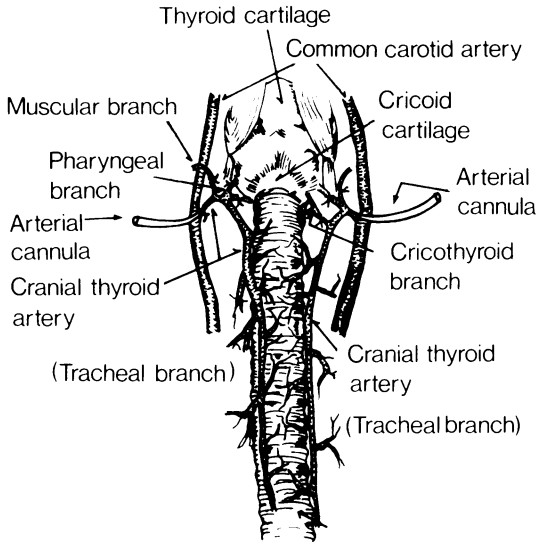

Figure 1 Arterial supply of the trachea of the dog. Arterial cannulae are inserted into the cranial thyroid arteries on both sides.

maintained by intravenous infusion of the same anaesthetic at a rate of 5 mg $kg^{-1}h^{-1}$.

The upper cervical region was incised in the midline, and the left and right cranial thyroid arteries, the main arteries supplying the upper portion of the trachea, and their branches were exposed and carefully dissected free. Muscular, pharyngeal and cricothyroid branches of the cranial thyroid arteries were all ligated, and branches supplying the trachea were left intact. Two arms of a Y-shape cannula were inserted into the left and right cranial thyroid arteries, as shown in Figure 1. Arterial blood from the right femoral artery was conducted to the Y-shape cannula *via* a peristaltic pump (Harvard Apparatus, Model 1215). Constant pressure perfusion was achieved by shunting a portion of blood through a Starling pneumatic resistance to the right femoral vein. The perfusion pressure was set at a value slightly lower than the mean systemic blood pressure at the beginning of perfusion and kept constant throughout the experiment. The perfusion pressure was monitored from a side arm of the perfusion circuit and the systemic blood pressure was measured from the femoral artery by pressure transducers (Nihon Kohden, MPU-0.5). The rate of blood flow through

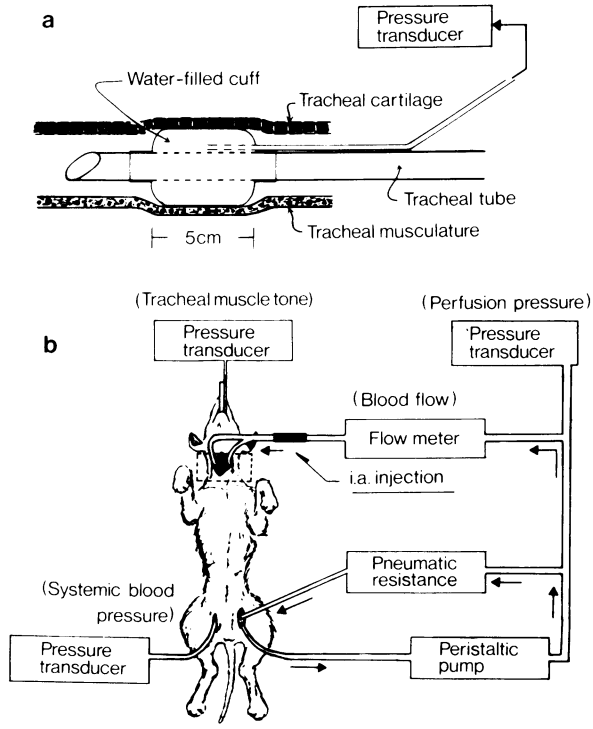

Figure 2 (a) Schematic illustration of the tracheal tube with a water-filled cuff attached for measurement of the tracheal intraluminal pressure. (b) Diagram of circuit for constant pressure perfusion of the tracheal vascular bed through the cranial thyroid arteries with blood from the femoral artery.

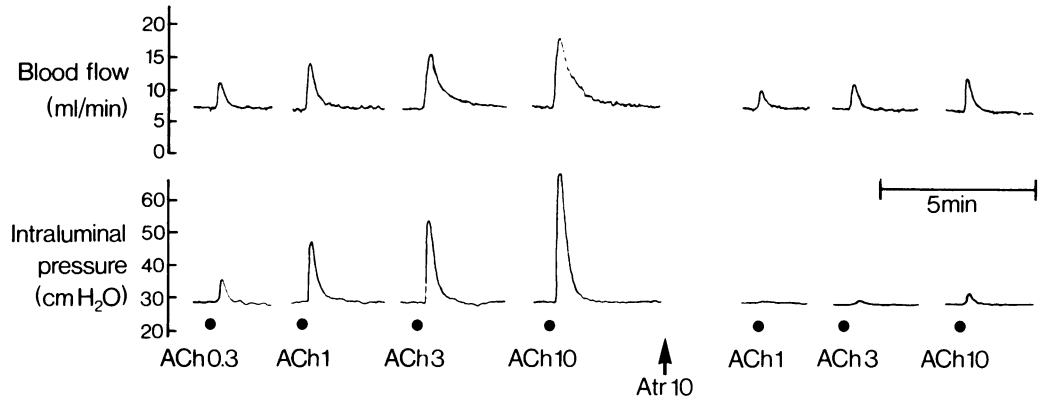

Figure 3 Responses of the tracheal vascular bed (blood flow rate) and musculature (intraluminal pressure) to intra-arterial acetylcholine (ACh) and antagonism by intra-arterial atropine (Atr). All doses in μg.

the arteries was measured with an electromagnetic flowmeter (Nihon Kohden, MF-46-3) situated just proximal to the Y-shape cannula. Just before the start of perfusion the animal was given intravenous heparin sodium, 500 units/kg, and 100 units/kg were added intravenously at hourly intervals. Responses of the tracheal smooth muscle were measured as changes in the intraluminal pressure of a water-filled cuff (5 cm in length) attached to a tracheal tube, which was introduced into the trachea *via* the mouth (Figure 2a). The water-filled cuff was connected to a pressure transducer (Nihon Kohden, MPU-0.1) through polyethylene tubing (Intramedic PE 100). The water-filled cuff was located from 3 to 8 cm caudal to the larynx. The volume of water in the cuff was adjusted initially to give resting intraluminal pressures of 25–35 cmH_2O. This pressure range was found to be suitable for the observation of both dilatation and constriction of the trachea. Recordings were made on an ink-writing rectigraph (San-ei Instrument, Rectiholiz 8s). The experimental set-up is illustrated schematically in Figure 2b.

In 16 dogs at the conclusion of experiment, 0.4% w/v indigo carmine in 0.9% w/v NaCl solution (saline) was injected intra-arterially and the area perfused was checked. The upper two thirds of the trachea from the larynx was found to be clearly stained by the dye. In addition, the ventral upper third of the oesophagus was also slightly stained.

Drugs used in this study were acetylcholine chloride (Daiichi), atropine sulphate (Merck), (−)-adrenaline (Merck), (−)-noradrenaline (Fluka), (−)-isoprenaline hydrochloride (Nikken Kagaku), (±)-propranolol hydrochloride (ICI), phentolamine methanesulphonate (Ciba), 5-hydroxytryptamine creatinine sulphate (Wako) and methysergide tartrate (Sandoz). Adrenaline and noradrenaline were dissolved in 0.01 N HCl. All other drugs were dissolved in saline. All drug solutions were diluted with saline to the desired concentrations. Drug solutions in a volume of 30 μl (in 4 s) or 100 μl (in 15 s) were injected by the use of microsyringes into the rubber tubing just proximal to the Y-shape cannula.

Doses of acetylcholine, adrenaline, noradrenaline and 5-hydroxytryptamine refer to their bases and of atropine, propranolol, phentolamine and methysergide to their salts.

Values in the text are arithmetic means ±s.e. (unless otherwise stated). The statistical significance of the differences between mean values was analysed with Student's *t*-test and expressed as *P* values.

Results

Basal values of main parameters under resting conditions

The mean systemic blood pressure was 134.9 ± 3.7 mmHg ($n=38$). The retrograde pressure measured by clamping the tubing just proximal to the side arm to the pressure transducer was 72.8 ± 2.4 mmHg ($n=28$). The retrograde flow from the arterial cannula disconnected from the tubing was 4.1 ± 0.2 ml/min ($n=28$). This was measured by the timed collection of blood in a graduated cylinder. The blood flow rate at a constant perfusion pressure of about 130 mmHg (127.5 ± 6.2 (s.d.) mmHg ($n=38$)) was 9.5 ± 0.5 ml/min ($n=38$). The preparation remained in good condition for 8 h or more.

Effects of acetylcholine on tracheal blood flow rate and smooth muscle tone

Single intra-arterial injections of acetylcholine (0.3–10 μg) produced dose-dependent increases in blood flow rate, *viz.*, vasodilatation and in intraluminal

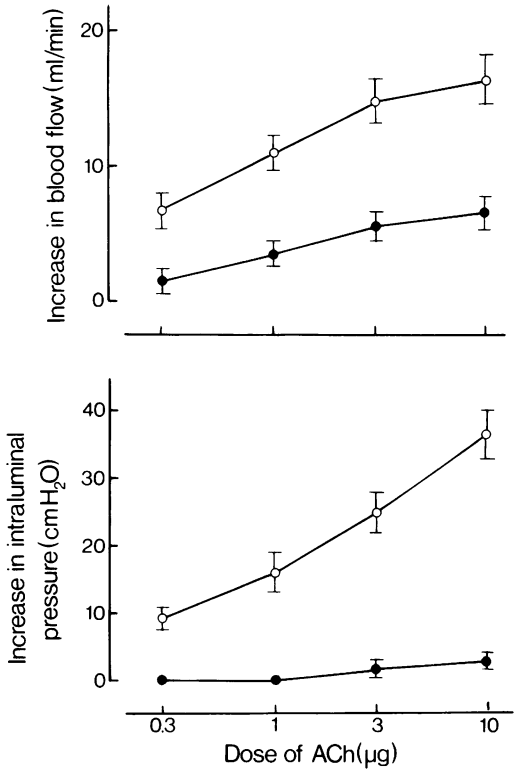

Figure 4 Dose-response curves for increase in rate of blood flow through and increase in intraluminal pressure of the trachea to intra-arterial acetylcholine before (○) and after (●) atropine (10 μg, i.a.). Each point represents the mean of 14 observations on 14 animals. Vertical bars show s.e. mean.

pressure, *viz.*, tracheal constriction. Figure 3 is typical of such experiments and Figure 4 shows dose-response curves for peak increases in the blood flow rate and intraluminal pressure. In some preparations, the tracheal constriction caused by acetylcholine was followed by tracheal dilatation. Intra-arterial injections of these doses of acetylcholine caused no systemic effect.

The vasodilatation and tracheal constriction caused by acetylcholine were antagonized by atropine, 10 μg intra-arterially ($P<0.01$) (Figures 3 and 4), the effect of atropine lasting for about 40 minutes. The tracheal dilatation following the constriction in response to acetylcholine observed in some preparations was also blocked by atropine.

Effects of (−)-isoprenaline, (−)-adrenaline and (−)-noradrenaline on tracheal blood flow rate and smooth muscle tone

Single injections of isoprenaline (0.03–1 μg, i.a.) caused dose-dependent increases in blood flow (vasodilatation) and decreases in intraluminal pressure (tracheal dilatation) (Figures 5 and 6). Adrenaline (0.1–1 μg, i.a.) and noradrenaline (0.3–3 μg, i.a.) produced vasoconstriction but like isoprenaline these two catecholamines caused tracheal dilatation in a dose-dependent manner (Figures 5 and 6). In only one of 13 preparations, including those used in preliminary experiments, 1 and 3 μg of noradrenaline increased intraluminal pressure (tracheal constriction) by about 12 and 20 cmH_2O, respectively. Relative potencies determined on the basis of doses producing a 10 cmH_2O decrease in the intraluminal pressure were in descending order: isoprenaline > adrenaline > noradrenaline, being approximately 1 : 1/3 : 1/12 (Figure 6). Adrenaline was more potent than noradrenaline in producing vasoconstriction ($P<0.05$) (Figure 6). Intra-arterial injections of these doses of the three catecholamines had no systemic effect.

Modification by (±)-propranolol and phentolamine of the effects of catecholamines

A single injection of propranolol (60 μg, i.a.) increased transiently (0.5–1 min) the blood flow rate by about 4.5 ml/min but had no effect on the tracheal intraluminal pressure. This dose of propranolol blocked the vasodilatation and tracheal dilatation caused by isoprenaline ($P<0.01$) and converted the tracheal dilatation in response to adrenaline and noradrenaline to constriction (Figures 5 and 6). In the presence of propranolol, adrenaline was 2–3 times as potent as noradrenaline in producing tracheal constriction ($P<0.05$). The tracheal constriction in response to adrenaline and noradrenaline after an intra-arterial injection of propranolol developed slowly in comparison with that to acetylcholine, as shown in Figure 5 (cf. Figure 3). The vasoconstriction caused by adrenaline and noradrenaline was not significantly different before and after propranolol ($P>0.6$) (Figure 6). The blocking effect of 60 μg of propranolol on the tracheal dilatation caused by isoprenaline, adrenaline and noradrenaline lasted for 30–40 minutes. This dose of propranolol did not modify the tracheal constriction caused by 10 μg of acetylcholine.

The tracheal constriction in response to adrenaline and noradrenaline in the presence of propranolol was almost abolished by a single intra-arterial injection of 200 μg of phentolamine ($P<0.01$), as shown in Figures 5 and 6. The vasoconstriction in response to adrenaline and noradrenaline was also antagonized by phentolamine ($P<0.05$) (Figures 5 and 6). The antagonism by phentolamine lasted for 50–60 minutes.

The tracheal constriction caused by adrenaline and noradrenaline after propranolol was not modified by either atropine (10 μg) or methysergide (3 μg); these doses abolished the tracheal constriction caused by

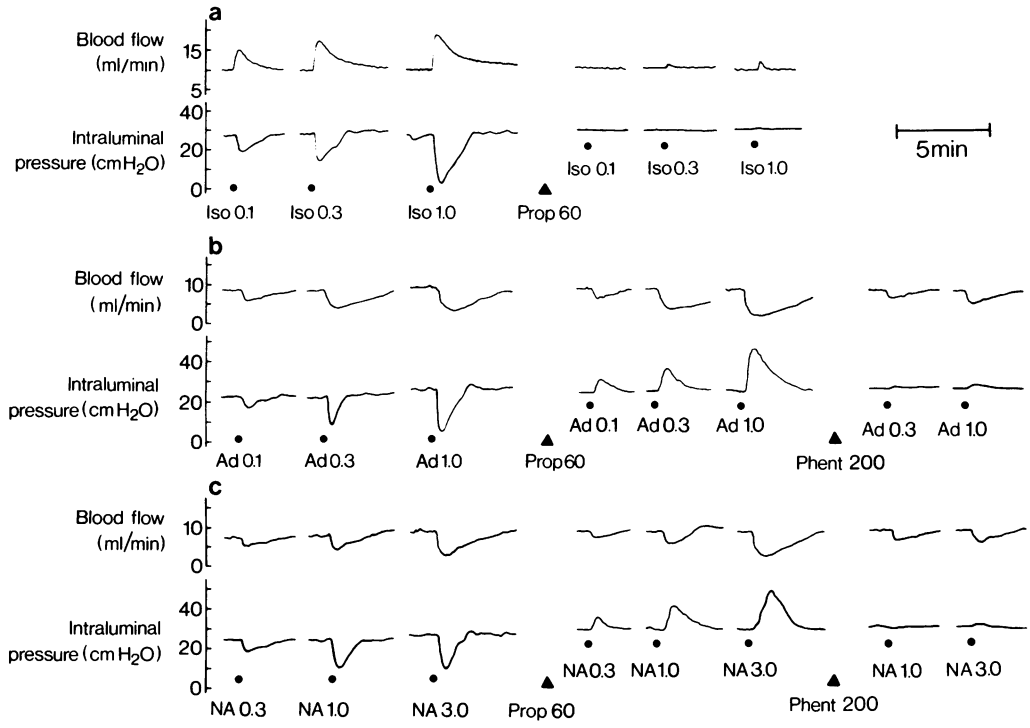

Figure 5 Responses of the tracheal vascular bed (blood flow rate) and musculature (intraluminal pressure) to (a) isoprenaline (Iso), (b) adrenaline (Ad) and (c) noradrenaline (NA), and modification by consecutive intra-arterial injections of propranolol (Prop) and phentolamine (Phent). All doses in μg.

acetylcholine (3 μg) and 5-hydroxytryptamine (3 μg), respectively.

Discussion

In the present experiments the trachea of the dog was perfused *in situ* with arterial blood through the cannulated cranial thyroid arteries after all the branches except those supplying the trachea had been ligated. However, the upper portion of the oesophagus is also supplied by arterial branches which supply the upper trachea (Miller, 1964). Thus, the question arises whether a blood flow response as observed in the present experiments represents the perfused tracheal vascular bed alone. Indigo-carmine injected intra-arterially during the perfusion stained the upper two thirds of the trachea and to a lesser degree the ventral half of the upper third of the oesophagus, demonstrating that the vascular response as recorded is mainly that of the tracheal vascular bed. Therefore, the arterially blood-perfused tracheal preparation of the dog is suitable for pharmacological analysis of drug action on the tracheal musculature and vasculature *in situ*.

Acetylcholine caused vasodilatation and tracheal constriction and these effects were antagonized by atropine, indicating that they were mediated *via* stimulation of muscarinic receptors.

Isoprenaline, adrenaline and noradrenaline produced relaxation of the tracheal smooth muscle and the order of their relative potencies are consistent with that of the β-adrenoceptor stimulant activity of the three catecholamines determined on the tracheal smooth muscle of the calf (Ariëns, 1967) and guinea-pig (Guirgis, 1969; Chahl, 1972). The tracheal relaxations in response to the three catecholamines were blocked by propranolol. This indicates that the relaxation of the tracheal smooth muscle of the dog is mediated through β-adrenoceptors as demonstrated in the guinea-pig (Foster, 1966; Takagi *et al.*, 1967; Everitt & Cairncross, 1969; Guirgis, 1969; Chahl & O'Donnell, 1971; Chahl, 1972). In the presence of propranolol, adrenaline and noradrenaline but not isoprenaline caused tracheal constriction. Adrenaline was more potent than noradrenaline. Thus, the relative potencies of the two catecholamines coincide with those found for α-adrenoceptor stimulant activity in the vas deferens of the rat (Ariëns, 1967) and in the isolated tracheal chain of the guinea-pig in the

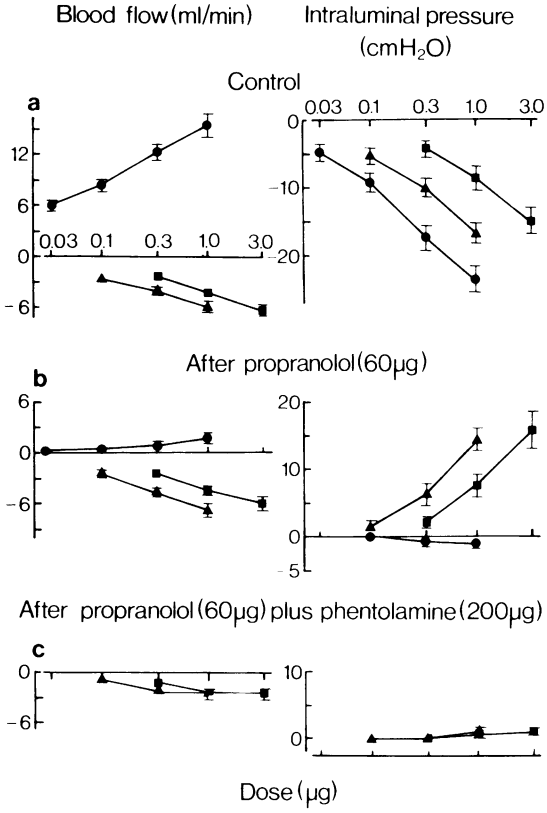

Figure 6 Dose-response curves for changes in rate of blood flow through (left side of figure) and intraluminal pressure (right side of figure) of the dog trachea to intra-arterial isoprenaline (●), adrenaline (▲) and noradrenaline (■), (a) before and (b) after a single injection of propranolol (60 μg, i.a.) and (c) after a further single injection of phentolamine (200 μg, i.a.). Each point represents the mean of 5–7 observations on 5–7 animals. Vertical bars show s.e. mean.

presence of propranolol (Everitt & Cairncross, 1969). The relative potencies also satisfy the criteria of Ahlquist (1966) which characterize α-adrenoceptors. The tracheal constriction was antagonized specifically by phentolamine. These results clearly indicate that the constrictor action of the two catecholamines on the dog trachea was mediated through excitatory α-adrenoceptors. The existence of the excitatory α-adrenoceptors in the tracheal smooth muscle has been demonstrated in the guinea-pig isolated tracheal preparations (Takagi *et al.*, 1967; Kasses *et al.*, 1968; Everitt & Cairncross, 1969; Fleisch *et al.*, 1970), although some investigators (Foster, 1966; Danko *et al.*, 1968; Rikimaru & Sudoh, 1971) failed to demonstrate it in the isolated guinea-pig trachea.

Isoprenaline caused tracheal vasodilatation, whereas adrenaline and noradrenaline caused tracheal vasoconstriction. The vasodilatation in response to isoprenaline was blocked specifically by propranolol, and the vasoconstriction in response to adrenaline and noradrenaline was antagonized specifically by phentolamine. This indicates that the vasodilatation was mediated through β-adrenoceptors whereas the vasoconstriction was mediated through α-adrenoceptors in the vasculature.

These results taken together indicate that inhibitory β-adrenoceptors predominate over excitatory α-adrenoceptors in the tracheal musculature and *vice-versa* in the tracheal vasculature of the dog.

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