Br. J. Pharmac. (1982), **75**, 433–440

DUAL EFFECTS OF CATECHOLAMINES ON PRE- AND POST-JUNCTIONAL MEMBRANES IN THE DOG TRACHEA

YUSHI ITO & KIKUO TAJIMA

Department of Pharmacology, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan

1 Effects of noradrenaline or isoprenaline on the membrane and contractile properties of the smooth muscle cell, or on the excitatory neuro-effector transmission in the dog trachea, *in vitro*, were observed by use of microelectrodes and double sucrose gap methods.

2 Noradrenaline ($< 5 \times 10^{-6}$ M) or isoprenaline ($< 5 \times 10^{-7}$ M) modified neither the membrane potential nor the membrane resistance. Increased concentrations of noradrenaline ($> 5 \times 10^{-5}$ M) depolarized and isoprenaline ($> 5 \times 10^{-7}$ M) hyperpolarized the membrane, and these actions were suppressed by phentolamine and propranolol respectively. Both catecholamines reduced the membrane resistance.

3 Noradrenaline (5×10^{-6} M) or isoprenaline (5×10^{-7} M) reduced the resting tension, raised the mechanical threshold required to produce the contraction and suppressed the amplitude of phasic contractions evoked by electrical depolarization of the membrane.

4 The action potential evoked by an outward current pulse in the presence of tetraethylammonium (TEA) was not affected by 5×10^{-6} M isoprenaline, while the mechanical response was markedly suppressed.

5 The excitatory junction potential (e.j.p.) evoked by electrical field stimulation was blocked by atropine. Noradrenaline (5×10^{-7} M) or isoprenaline (5×10^{-8} M) suppressed the amplitude of e.j.p. with no change in the membrane potential or input membrane resistance. Depression in the amplitude of e.j.ps produced by noradrenaline or isoprenaline reduced the amplitude of phasic contractions evoked by e.j.ps.

6 These inhibitory actions of the catecholamines on mechanical responses and on e.j.ps were suppressed by pretreatment with propranolol (4×10^{-6} M).

7 Dog tracheal smooth muscles are innervated by cholinergic excitatory and adrenergic inhibitory systems. Electrical field stimulation produced excitation of both cholinergic and adrenergic nerve fibres, and propranolol (4×10^{-6} M) enhanced the amplitude of e.j.p. generated by excitation of cholinergic nerves when repetitive stimulation (10 stimuli at 20 Hz) was used, but not the amplitude of the e.j.p. evoked by a single stimulus.

8 5-Hydroxytryptamine (6×10^{-6} M) produced a tonic contracture of the dog trachea. After pretreatment with atropine (4×10^{-6} M), field stimulation (50 μs in duration and repetitive stimuli at 20 Hz) induced reversal of the contracture induced by 5-hydroxytryptamine and this was abolished by propranolol (5×10^{-6} M).

9 These results indicate that endogenous or exogenous catecholamines, in relatively low concentrations, predominantly activate β-adrenoceptors in the pre- and post-junctional membrane in the dog trachea, and induce muscle relaxation.

Introduction

As early as 1910, sympathomimetic agents were used to treat patients with bronchial asthma (Barger & Dale, 1910). Noradrenaline was largely replaced by isoprenaline because of its relative lack of activity on α-adrenoceptors and agents such as metaproterenol, salbutamol or terbutaline have been used to treat bronchial asthma (see for example Widdicombe, 1963). Despite the wide-spread daily administration of these compounds to patients with bronchial asthma, there is little documentation on the cellular mode of actions of sympathomimetic agents on bronchial or tracheal smooth muscle tissues.

The present experiments were carried out in an attempt to investigate the cellular actions of catecholamines on the excitation-contraction coupling in tracheal smooth muscle cells and the cholinergic neuro-effector transmission in the dog trachea. This particular preparation was used because the

0007-1188/82/030433-08 $01.00

electrical membrane properties of the smooth muscle and neuro-effector transmission have already been studied in detail (Suzuki, Morita, Kuriyama, 1976; Ito & Tajima, 1981a,b).

Methods

Adult mongrel dogs of either sex weighing 10–15 kg were anaesthetized with pentobarbitone (30 mg/kg, i.v.). Segments of cervical trachea were excised, and a dorsal strip of transversely running smooth muscle fibre was separated from the cartilage. Circular muscle strips were cut, 2.0–2.5 mm wide and approx. 1.5 cm long, for application of the double sucrose gap method. For intracellular recording of the membrane potential from a single cell, thin strips of tissue 10–15 mm in length, 4–5 mm in width and 0.3–0.4 mm thick were used. The preparation was bathed in a modified Krebs solution with the following ionic composition (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134.0, $H_2PO_4^-$ 1.2, HCO_3^- 15.5 and glucose 11.5. The solution was aerated with 97% O_2 and 3% CO_2 and pH was adjusted to 7.3–7.4.

The apparatus and experimental procedures for the microelectrode and the double sucrose gap methods were the same as those described by Ito & Tajima (1981a). The following drugs were used; indomethacin (Sigma Ltd), atropine sulphate (Daiichi), (−)-isoprenaline hydrochloride (Nikken Chemical Co., Ltd), noradrenaline (Sankyo), phentolamine (CIBA-Geigy) and (−)-propranolol (Sumitomo).

Results

Effects of noradrenaline or isoprenaline on the resting membrane potential of the smooth muscle cells of dog trachea

Isoprenaline (5×10^{-9}–5×10^{-7} M) had no effects on the resting membrane potential of the smooth muscle cells of dog trachea, i.e., the resting membrane potential in Krebs solution was -59.8 ± 1.0 mV (mean ± s.d., $n = 30$), whereas in the presence of isoprenaline (5×10^{-7} M), it was -60.1 ± 1.3 mV ($n = 25$). On the other hand, with increased concentrations of isoprenaline (5×10^{-6} M), the membrane was hyperpolarized from -58.2 ± 1.2 mV ($n = 30$) to -65.4 ± 2.2 mV ($n = 25$, $P < 0.05$), and in 5×10^{-5} M from -59.1 ± 2.0 mV ($n = 30$) to -65.7 ± 2.1 mV ($n = 30$, $P < 0.05$). The membrane

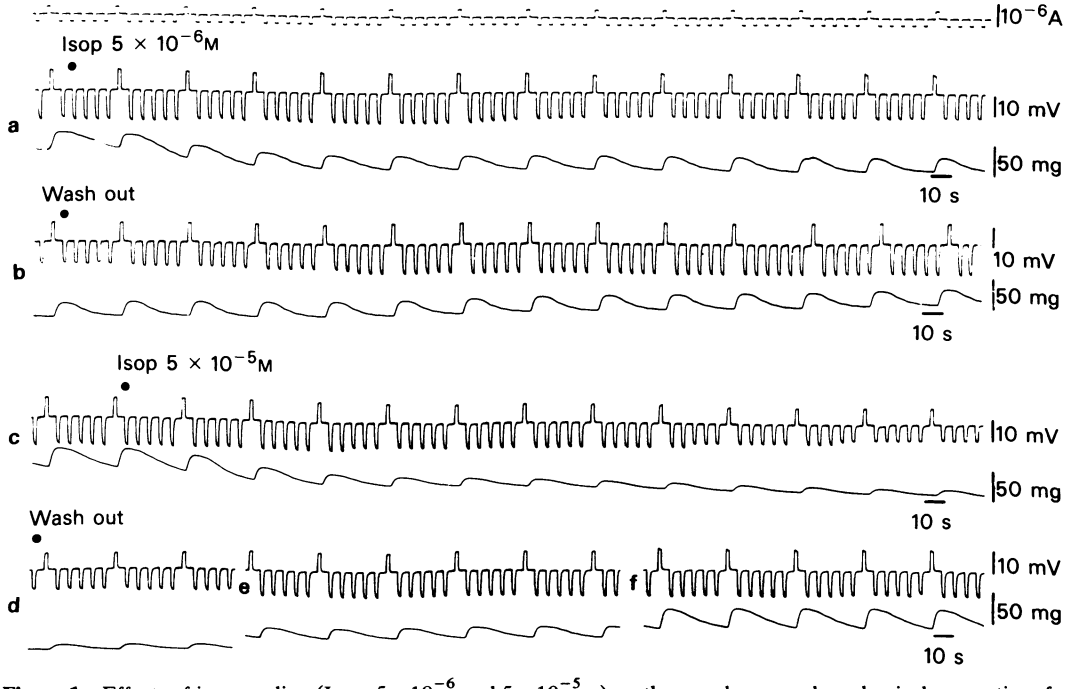

Figure 1 Effects of isoprenaline (Isop, 5×10^{-6} and 5×10^{-5} M) on the membrane and mechanical properties of the dog tracheal smooth muscle cells observed using the double sucrose gap method. Dots indicate the application or the withdrawal of the drug. (a) and (b), (c) and (d) are continuous records. Time lag between (d) and (e), (e) and (f) was about 5 min.

hyperpolarizations induced by isoprenaline were abolished by pretreatment with propranolol (10^{-5} M).

Noradrenaline (5×10^{-9}–5×10^{-6} M) also had no effect on the membrane potential during prolonged treatment (up to 1 h). However, noradrenaline 5×10^{-5} M, significantly depolarized the membrane from -58.3 ± 1.8 mV ($n = 30$) to -52.9 ± 1.8 mV ($n = 25$, $P < 0.01$). This depolarization was completely suppressed by phentolamine (2×10^{-5} M).

Effects of noradrenaline or isoprenaline on the membrane and mechanical properties

We used the double sucrose gap method to assess the effects of catecholamines on the membrane and mechanical properties of the smooth muscle cells.

Isoprenaline (5×10^{-7} M) decreased the resting tension without significantly affecting the resting membrane potential or input resistance, as measured from the amplitude of electrotonic potentials induced by square wave pulses (2 s duration). However, with increased concentrations (5×10^{-6} M), isoprenaline decreased the input resistance of the membrane and greatly reduced the resting tension (Figure 1). The relative reductions in the input membrane resistance observed by the application of 5×10^{-6} or 5×10^{-5} M isoprenaline were $22 \pm 5\%$ ($\pm$s.d., $n = 5$) or $37 \pm 5\%$ ($\pm$s.d., $n = 4$) of the control value, respectively.

Similar experiments were also done with noradrenaline. After pretreatment with phentolamine (2×10^{-5} M), noradrenaline (2.5×10^{-5} M) significantly reduced the input membrane resistance (to $75 \pm 6\%$ of the control value) and also reduced the resting tension.

To observe the effects of catecholamines on the mechanical properties of tracheal muscles, the relationship between membrane depolarization and evoked contraction was observed in the presence or absence of catecholamines. Outward current pulses (2 s in duration) did not produce an active membrane response, however when a depolarization exceeded 7 mV, a contraction was evoked. The amplitude of contraction induced by electrical depolarization increased in proportion to the applied current intensity over the range used.

Isoprenaline in the presence of phentolamine (2×10^{-5} M) suppressed the amplitude of contraction at any given depolarization of the membrane produced by application of outward current pulses and

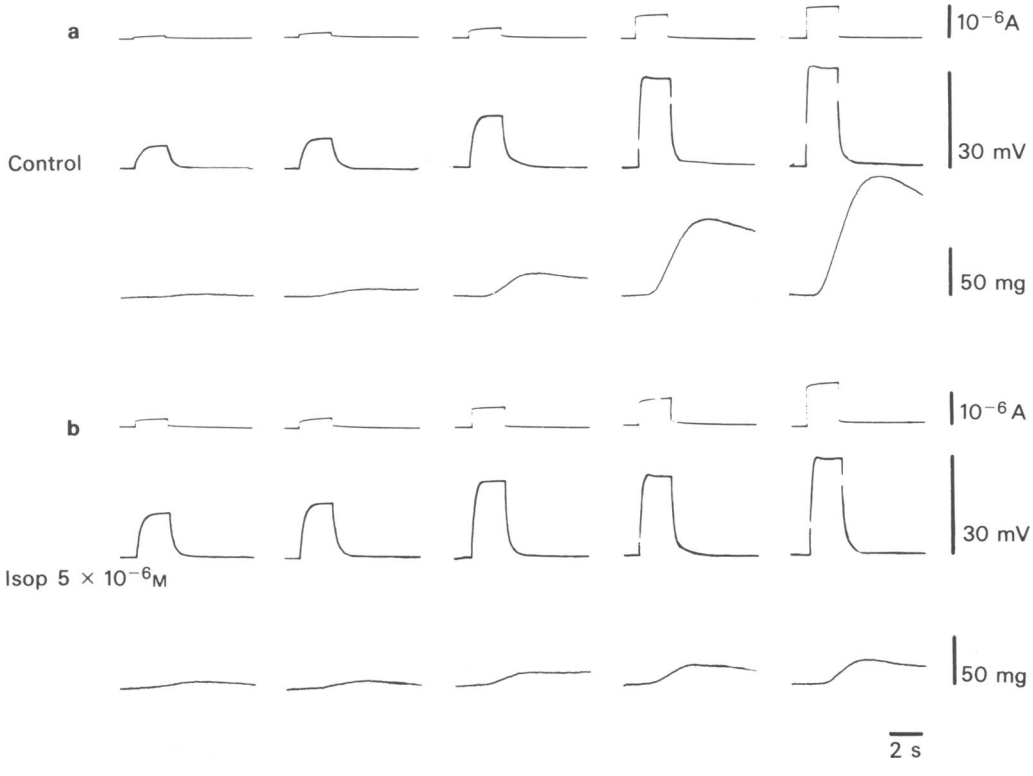

Figure 2 Depolarization-contraction relationship observed in the presence or absence of isoprenaline (Isop) after pretreatment with phentolamine (2×10^{-5} M). (a) Control; (b) in the presence of isoprenaline 5×10^{-6} M.

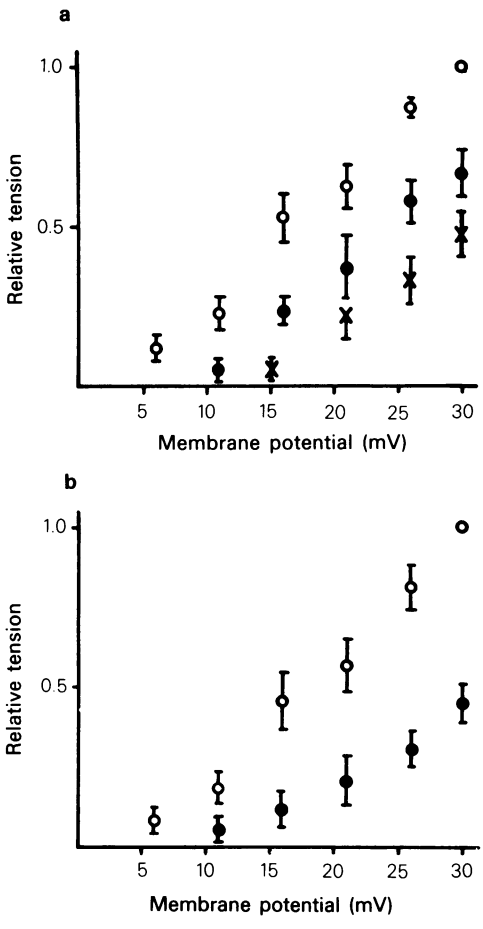

Figure 3 Depolarization-contraction relationship observed in the presence or absence of catecholamines after pretreatment with phentolamine (2×10^{-5} M). (a) (○) Control; (●) isoprenaline 5×10^{-7} M; (×) isoprenaline 5×10^{-6} M. (b) (○) Control; (●) noradrenaline 2.5×10^{-6} M.

raised the depolarization required to produce the contraction. Figure 2 shows examples of the effects of 5×10^{-6} M isoprenaline on the depolarization-induced contraction. Figure 3a represents the relationship between the depolarization and contraction in the presence or absence of isoprenaline. The amplitude of the contraction evoked by 30 mV depolarization in Krebs solution was registered as a relative contraction of 1.0. In the presence of isoprenaline (5×10^{-7} M), the membrane potential remained unaffected but the minimum depolarization required to produce tension development was increased from 7 mV to 11 mV, and in 5×10^{-6} M, from 7 mV to 15 mV. The amplitude of contraction evoked by 30 mV depolarization was reduced to 66% or 47% of the control value in the presence of 5×10^{-7} or 5×10^{-6} M of isoprenaline, respectively. Similar experiments were carried out in which noradrenaline was applied after pretreatment with phentolamine (2×10^{-6} M). Noradrenaline (2.5×10^{-6} M) increased the minimum depolarization required to produce tension development from 7 mV to 12 mV, and the amplitude of contraction at 30 mV depolarization was reduced to 46 ± 6% of the control value (Figure 3b). These effects of the catecholamines on the electrical and mechanical properties of the tracheal smooth muscle cells were abolished by pretreatment with propranolol (2×10^{-5} M), but not by phentolamine (2×10^{-5} M).

Effects of isoprenaline on electrical and mechanical properties in the presence of tetraethylammonium

In the dog tracheal muscle, tetraethylammonium (TEA) suppressed the rectifying property of the membrane, increased the membrane resistance, and the spike evoked by outward current pulses (Suzuki *et al.*, 1976). The effects of isoprenaline on the tension development induced by a spike in the presence of TEA were observed. With TEA (5×10^{-3} M), outward current pulses produced a spike with a large phasic tension development when the depolarization exceeded 10 mV (about 10 times larger than the evoked contraction by 30 mV depolarization produced by outward current pulses in Krebs solution). In the presence of TEA (5×10^{-3} M), application of isoprenaline (5×10^{-6} M) reduced the resting tension and suppressed the amplitude of evoked contrac-

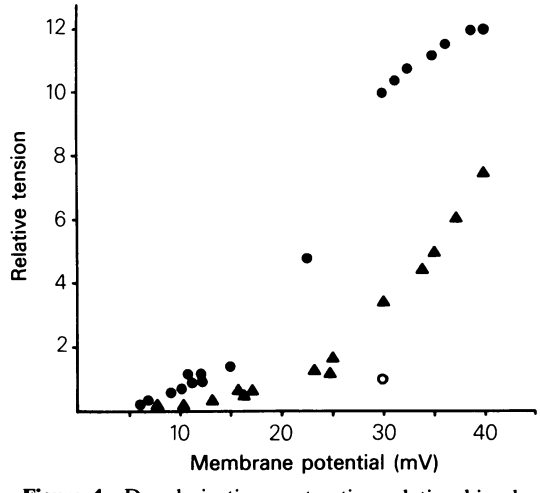

Figure 4 Depolarization-contraction relationship observed in the presence of tetraethylammonium (TEA) or TEA with isoprenaline, the relative amplitude of contraction recorded by 30 mV depolarization in the Krebs solution was taken as 1.0. (○) Control; (●) TEA 5×10^{-3} M; (▲) TEA 5×10^{-3} M plus isoprenaline 5×10^{-6} M.

tions, but did not affect the threshold depolarization required for spike generation nor the maximum rate of rise of the spike (TEA alone; 5.1 ± 0.8 V/s; in the presence of TEA and isoprenaline; 5.0 ± 0.6 V/s, $n = 5$, ±s.d.). Figure 4 shows the depolarization-contraction relationship in the presence of TEA or TEA with isoprenaline. The relative amplitude of contraction evoked by 30 mV depolarization in Krebs solution was considered as 1.0. In the presence of TEA, isoprenaline suppressed the depolarization-contraction relationship, but did not affect the minimum membrane depolarization required to produce contractions.

Effects of catecholamines on the cholinergic transmission

Under certain conditions, cholinergic transmission can be modified by catecholamines (Hidaka & Kuriyama 1969; Kuba & Tomita 1971; Vermeire & Vanhoutte 1979). As the dog tracheal smooth muscles are innervated by cholinergic and adrenergic nerve fibres (Cabezas, Graf & Nadel 1971; Suzuki *et al.*, 1976), we examined the effects of catecholamines on the excitatory neuro-effector transmission. For this purpose, excitatory junction potentials (e.j.ps) and phasic tension developments were recorded using the double sucrose gap method. To obtain e.j.ps with a constant amplitude by a given stimulus condition, indomethacin (3.6×10^{-5} M) was used throughout the experiments (Ito & Tajima 1981a,b).

After pretreatment with phentolamine (2×10^{-5} M), field stimulation (50 μs in duration) applied by the double sucrose gap method produced e.j.p. followed by phasic contraction. Figure 5 shows the reduction by noradrenaline or isoprenaline of the amplitude of e.j.ps and phasic contractions. In the presence of 5×10^{-8} M isoprenaline, the amplitude of e.j.p. was reduced to $81 \pm 6\%$ (±s.d., $n = 5$) of the control value with no significant changes in the membrane potential and input membrane resistance. Noradrenaline (5×10^{-8} M) did not modify the amplitude of the e.j.p., but this was significantly reduced to $78 \pm 5\%$ of the control value by 5×10^{-7} M ($P < 0.05$). At higher concentrations (5×10^{-6}–2.5×10^{-4} M), both agents almost abolished the generation of e.j.ps and phasic tension development.

The relationship between catecholamine concentrations and the changes in amplitude of e.j.ps or in the input membrane resistance was measured in the presence of the α-blocker, phentolamine (2×10^{-5} M). Low concentrations of noradrenaline or isoprenaline reduced the amplitude of e.j.ps with no change in the input membrane resistance (Figure 6). These inhibitory actions of catecholamines on the e.j.p. and on mechanical responses were antagonized by pretreatment of the muscles with propranolol (10^{-6} M).

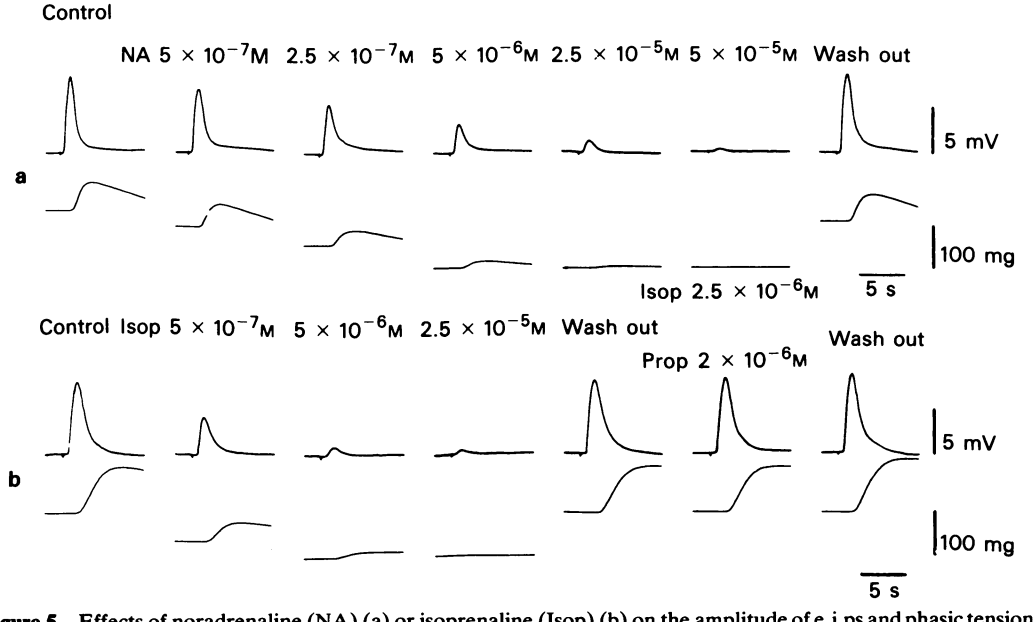

Figure 5 Effects of noradrenaline (NA) (a) or isoprenaline (Isop) (b) on the amplitude of e.j.ps and phasic tension developments in the presence of indomethacin (3.6×10^{-5} M) and phentolamine (2×10^{-5} M). Effects of propranolol (2.5×10^{-6} M) on the action of isoprenaline was also observed after the pretreatment with phentolamine (b). Field stimulation (50 μs in duration) was applied to evoke e.j.p.

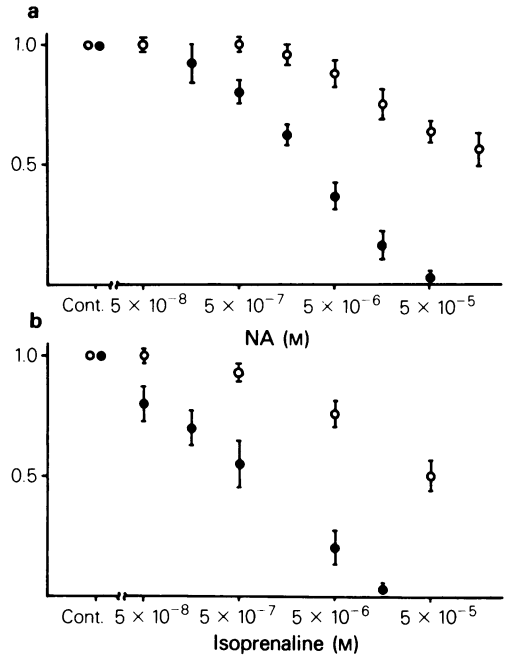

Figure 6 Relationship between the concentration of noradrenaline (NA) (a) or isoprenaline (b) and the relative amplitude of e.j.p. (●) or relative value of the input membrane resistance (○). The relative amplitude of e.j.p. in normal Krebs solution was taken as 1.0, and the relative amplitude of the electrotonic potentials induced by the square wave pulses (2 s duration) in normal Krebs solution was taken as 1.0.

Effects of propranolol on the e.j.p.

Field stimulation (50 μs duration and repetitive stimuli at 20 Hz) to the tissue excited both cholinergic and adrenergic nerve fibres in the dog trachea, and produced e.j.ps accompanied by biphasic mechanical responses, i.e. the initial phasic contraction and subsequently longer lasting relaxation. These electrical and mechanical responses were suppressed by either atropine (4×10^{-6} M) or propranolol (5×10^{-6} M), respectively. To clarify whether or not the activation of adrenergic fibres affects the cholinergic neuroeffector transmission, we measured the amplitude of e.j.ps before and during application of propranolol, under various conditions of stimulation. When a single stimulus (50 μs duration) was used, the amplitude of the e.j.p. was not affected by treatment with propranolol (5×10^{-6} M). However, the amplitude of the e.j.p. was increased to 114 ± 4% (± s.d., $n = 5$, $P < 0.05$) in comparison to that of the control value when repetitive stimulation (10 stimuli at 20 Hz) was applied to evoke e.j.ps in the presence of propranolol (5×10^{-6} M).

Effects of adrenergic nerve stimulation on the contracture evoked by 5-hydroxytryptamine

To observe the effects of adrenergic nerve stimulation on the contracture of tracheal smooth muscles, 5-hydroxytryptamine (5-HT) was used. As shown in Figure 7, 5-HT (6×10^{-6} M) evoked muscle contracture with a steady increase in tone, up to a certain level and without fluctuations. Field stimulation (50 μs duration and repetitive stimuli at 20 Hz) during the tonic contracture produced an initial phasic contraction and subsequent relaxation. The amplitude of contraction or relaxation was increased in proportion to the number of stimuli at a constant stimulus intensity and frequency. After pretreatment with atropine, field stimulation induced rapid relaxations following a gradual increase in the muscle tone (Figure 7b, c and d). The amplitude of relaxation was dependent on the number of stimuli at the constant stimulus intensity, and 20 stimuli at 20 Hz produced relaxation of the muscle, i.e., the muscle tone was reduced to about 40% of the maximum contraction induced by 5-HT (6×10^{-6} M). These relaxations were abolished by propranolol (4×10^{-6} M) (Figure 7e).

Discussion

Dog tracheal smooth muscles are innervated by cholinergic excitatory and adrenergic inhibitory systems, but not by nonadrenergic noncholinergic inhibitory systems (Cabezas *et al.*, 1971; Suzuki *et al.*, 1976; Ito & Tajima, 1981a). *In vivo* experiments in the dog or the rabbit revealed that stimulation of sympathetic nerves dilates airways when vagal bronchoconstriction is present (Cabezas *et al.*, 1971), and that injection of isoprenaline reduces the bronchoconstriction induced by application of histamine (Mills, Sellick & Widdicombe, 1969). In the present experiments, low concentrations of noradrenaline (5×10^{-6} M) or isoprenaline (5×10^{-7} M) reduced the resting tension, elevated the mechanical threshold required to produce the contraction and suppressed the contraction evoked by electrical stimulations, while the above concentrations of these agents had no effects on the membrane properties. Furthermore, stimulation of endogenous adrenergic fibres produced relaxant effects on the contracture evoked by 5-HT.

It is known that the inhibitory action of catecholamines on visceral smooth muscles can be mediated by α or β-adrenoceptors, but there are distinct differences in the mechanisms by which the inhibitory effects are produced. For example, in the taenia coli, isoprenaline suppresses membrane activities and contraction with no remarkable changes in the membrane potential or membrane resistance

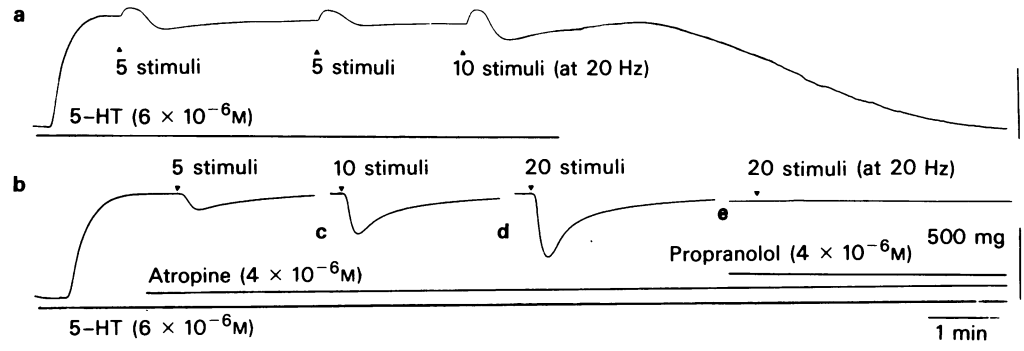

Figure 7 Effects of field stimulations (50 μs in duration and repetitive stimuli at 20 Hz) on the mechanical properties of the dog trachea after pretreatment with 5-hydroxytryptamine (5-HT 6×10^{-6} M); (a) field stimulation evoked biphasic changes in the muscle tone, i.e. initial phasic contraction and subsequently generated long lasting relaxation; (b), (c) and (d) after pretreatment with 5-HT (6×10^{-6} M) and atropine (4×10^{-6} M), field stimulation (5, 10 and 20 stimuli at 20 Hz)-evoked phasic relaxation of the muscle; (e) propranolol (4×10^{-6} M) abolished these effects of field stimulation on the muscle tone.

(Bülbring & Tomita 1969a,b). Similarly, in the canine coronary artery, catecholamines had no effect on the electrical membrane properties, but these agents did modify the mechanical responses, i.e. isoprenaline and a low concentration of phenylephrine reduced the development of the resting and evoked tension (Ito, Kitamura & Kuriyama, 1980). However, in the porcine coronary artery, noradrenaline and isoprenaline hyperpolarized the membrane and reduced the membrane resistance, resulting in suppression of the relationship between the depolarization-contraction coupling (Ito, Kitamura & Kuriyama, 1979).

In smooth muscle cells, the development of tension is determined by the concentration of ionized free calcium in the cytoplasm, and relaxation occurs when the internal free calcium falls below 10^{-7} M (Saida & Nonomura, 1979). Recently, Bülbring & Den Hertog (1980) reported that isoprenaline increased ^{45}Ca efflux by about 20%, while ^{45}Ca influx remained unchanged. They postulated that β-action of catecholamines may stimulate an electrogenic calcium-extrusion pump, resulting in a reduction of the size of phasic contractions or in the resting tension, indicating a lowering of the free Ca concentration in the cytoplasms.

In the present experiments, isoprenaline did not affect the maximum rate of rise of spike in the presence of TEA, but did reduce the resting and phasic tension development. These observations suggest that isoprenaline does not affect voltage-dependent inward calcium-currents, but does reduce the amount of intracellular free calcium ions in the presence or absence of TEA.

It is likely that potent relaxant effects of endogenous or exogenous catecholamines on the dog tracheal muscle observed in the present experiments may be induced through acceleration of the calcium extrusion and/or sequestration of free calcium ions within the cell.

In the isolated bronchial smooth muscle of the dog, noradrenaline or isoprenaline was found to inhibit significantly the mechanical response induced by electrical nerve stimulation to a greater extent than that seen with the application of exogenous acetylcholine, and propranolol abolished the inhibition in both cases (Vermeire & Vanhoutte, 1979). Furthermore, propranolol augmented the contractile response to electrical nerve stimulation but not that to acetylcholine. From these indirect observations, Vermeire & Vanhoutte (1979) suggested that in the canine bronchi, catecholamines inhibit the cholinergic neuro-effector transmission, and induce dilatation during bronchoconstriction caused by increased cholinergic nerve activity. In the present experiments, low concentrations of catecholamines (5×10^{-8}–5×10^{-7} M) reduced the amplitude of e.j.ps with no changes in membrane potential and membrane resistance, and this inhibitory action was abolished by pretreatment with propranolol. The amplitude of the contracture of the canine bronchi induced by exogenous acetylcholine was little affected by the application of low doses of catecholamines, indicating that the sensitivity of the smooth muscle cells to acetylcholine was not affected by catecholamines (Vermeire & Vanhoutte, 1979). Therefore, it is plausible that the sites of action of catecholamines are also in the nerve terminals where the release of acetylcholine takes place. In this case the nerve terminals of the vagus in the dog tracheal muscle tissue possess β-receptors and the activation of these receptors probably results in suppression of transmitter release.

The dual actions of catecholamines on the pre- and

post-junctional β-adrenoceptors in the smooth muscle tissue of the trachea may explain their potent bronchodilator effects.

We are grateful to Prof. H. Kuriyama for continuous encouragement and to M. Ohara for critical reading of the manuscript.

References

BARGER, G. & DALE, H.H. (1910). Chemical structure and sympathomimetic action of amines. *J. Physiol*, **41**, 19–59.

BÜLBRING, E. & DEN HERTOG, A. (1980). The action of isoprenaline on the smooth muscle of the guinea-pig taenea coli. *J. Physiol.*, **304**, 277–296.

BÜLBRING, E. & TOMITA, T. (1969a). Increase of membrane conductance by adrenaline in the smooth muscle of guinea-pig taenea coli. *Proc. R. Soc. B.*, **172**, 89–102.

BÜLBRING, E. & TOMITA, T. (1969b). Suppression of spontaneous spike generation by catecholamines in the smooth muscle of the guinea-pig taenia coli. *Proc. R. Soc. B.*, **172**, 103–119.

CABEZAS, G.A., GRAF, P.D. & NADEL, J.A. (1971). Sympathetic versus parasympathetic nervous regulation of airways in dogs. *J. appl. Physiol.*, **31**, 651–655.

HIDAKA, T. & KURIYAMA, H. (1969). Effects of catecholamines on the cholinergic neuromuscular transmission in fish red muscle. *J. Physiol.*, **201**, 61–71.

ITO, Y., KITAMURA, K. & KURIYAMA, H. (1979). Effects of acetylcholine and catecholamines on the smooth muscle cell of the porcine coronary artery. *J. Physiol.*, **294**, 595–611.

ITO, Y., KITAMURA, K. & KURIYAMA, H. (1980). Nitroglycerine and catecholamines action on smooth muscle cells of the canine coronary artery. *J. Physiol.*, **309**, 171–183.

ITO, Y. & TAJIMA, K. (1981a). Actions of indomethacin and prostaglandins on neuro-effector transmission in the dog trachea. *J. Physiol.*, **319**, 379–392.

ITO, Y. & TAJIMA, K. (1981b). Spontaneous activities in the trachea of dogs treated with indomethacin: an experimental model for aspirin-related asthma. *Br. J. Pharmac.*, **73**, 563–371.

KUBA, K. & TOMITA, T. (1971). Noradrenaline action on nerve terminal in the rat diaphragm. *J. Physiol.*, **217**, 19–31.

MILLS, J.E., SELLICK, H. & WIDDICOMBE, J.G. (1969). Activity of lung irritant receptors in pulmonary microembolism, anaphylaxis and drug-induced bronchoconstrictions. *J. Physiol.*, **203**, 337–357.

SAIDA, K. & NONOMURA, Y. (1979). Characteristics of Ca^{2+}- and Mg^{2+}-induced tension development in chemically skinned smooth muscle fibers. *J. gen. Physiol.*, **72**, 1–14.

SUZUKI, H., MORITA, K. & KURIYAMA, H. (1976). Innervation and properties of the smooth muscle of the dog trachea. *Jap. J. Physiol.*, **26**, 303–320.

VERMEIRE, P.A. & VANHOUTTE, P.M. (1979). Inhibitory effects of catecholamines in isolated canine bronchial smooth muscle. *J. appl. Physiol.*, **46**, 787–791.

WIDDICOMBE, J.G. (1963). Regulation of tracheobronchial smooth muscle. *Physiol. Rev.*, **43**, 1–37.

(Received May 5, 1981.
Revised November 12, 1981.)