

# Pre- and post-junctional actions of procaterol, a $\beta_2$ -adrenoceptor stimulant, on dog tracheal tissue

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- 1 The effects of procaterol, a  $\beta_2$ -adrenoceptor agonist, on excitatory neuro-effector transmission in the dog trachea were investigated and the findings were compared to those seen with isoprenaline, with microelectrode, double sucrose gap and tension recording methods.
- 2 Procaterol ( $10^{-10}$ – $10^{-9}$  M) and isoprenaline ( $10^{-9}$  M) had no effect on the resting membrane potential or on the input resistance of the smooth muscle cells of dog trachea. However with increased concentrations ( $>10^{-8}$  M), these agents hyperpolarized the membrane and decreased the input resistance of the membrane.
- 3 Procaterol ( $10^{-10}$ – $10^{-7}$  M) and isoprenaline ( $10^{-9}$ – $10^{-7}$  M) dose-dependently reduced the amplitude of the twitch contractions evoked by field stimulation in the combined presence of indomethacin ( $10^{-5}$  M) and guanethidine ( $10^{-6}$  M). In parallel with actions on twitch contractions, procaterol ( $10^{-10}$ – $10^{-7}$  M) and isoprenaline ( $10^{-9}$ – $10^{-7}$  M) reduced the amplitude of the excitatory junction potentials (e.j.ps), evoked by single pulse field stimulation in the dog trachea.
- 4 Procaterol ( $10^{-8}$  M) had no effect on the post-junctional response of smooth muscle cells to exogenous acetylcholine (ACh) ( $10^{-7}$ – $10^{-6}$  M).
- 5 Pretreatment with ICI-118551, a  $\beta_2$ -adrenoceptor blocking agent, reduced the inhibitory action of procaterol on the amplitude of twitch contractions evoked by field stimulations in the dog trachea.
- 6 These results indicate that procaterol in low concentrations has a prejunctional action inhibiting the excitatory neuro-effector transmission in addition to a postsynaptic action, presumably by suppressing transmitter release from the vagus nerve terminals through  $\beta_2$ -adrenoceptors in the dog tracheal tissue. The pre- and post-junctional actions of procaterol explain its potent bronchodilator effects in clinical use.

## Introduction

Earlier work suggested that exogenously applied catecholamines have inhibitory actions on the pre- and post-junctional membranes in dog tracheal tissue. Low concentrations of noradrenaline or isoprenaline reduced the resting tension of the smooth muscle cells, elevated the electrical threshold required to produce contraction and suppressed acetylcholine release from the vagus nerve terminals, through activation of  $\beta$ -adrenoceptors (Ito & Tajima, 1982).

Procaterol, a  $\beta$ -adrenoceptor stimulant, has a high potency and selectivity for tracheal smooth muscle tissues (Himori & Taira, 1977; Takayanagi *et al.*, 1977; Yabuuchi *et al.*, 1977; Yamashita *et al.*, 1978; Tei *et al.*, 1979; Delhaye *et al.*, 1983; Fujiwara *et al.*, 1988). However, there is little documentation on the pre-junctional actions of this chemical in airway smooth muscle tissues.

The pre-junctional effects of procaterol, and the subtype of  $\beta$ -adrenoceptors located in the vagus nerve terminals in the dog trachea were investigated by recording tension development and excitatory junction potentials (e.j.ps) evoked by nerve stimulation. For comparison, the effects of isoprenaline on the twitch and e.j.p. amplitude were recorded.

## Methods

Mongrel dogs of either sex, weighing 10–15 kg, were anaesthetized by an i.v. injection of pentobarbitone ( $30 \text{ mg kg}^{-1}$ ). Segments of cervical trachea were excised and a strip of transversely running smooth muscle tissue was separated from the cartilage. The mucosa and adventitial areolar tissues were carefully removed. The tracheal smooth muscle was cut into

sections 2.0–2.5 mm wide and about 20 mm long for double sucrose gap experiments or into strips 5–7 mm long, 1–2 mm wide and 0.3–0.4 mm thick for tension or microelectrode recordings.

These preparations were bathed in a modified Krebs solution of the following ionic composition (mM):  $\text{Na}^+$  137.4,  $\text{K}^+$  5.9,  $\text{Mg}^{2+}$  1.2,  $\text{Ca}^{2+}$  2.5,  $\text{Cl}^-$  134.0,  $\text{H}_2\text{PO}_4^-$  1.2,  $\text{HCO}_3^-$  15.5 and glucose 11.5. The solution was aerated with 97%  $\text{O}_2$  and 3%  $\text{CO}_2$  and the pH was adjusted to 7.3–7.4.

For tension measurement, the tissue was mounted in a 1 ml organ bath through which the test solution flowed continuously at a temperature of 35°C. The preparation was mounted vertically and the ends were tied with silk thread. One end of the strip was tied to a mechanotransducer (Nihon-Kohden Ltd., RCA-5734) and the other end to a hook at the bottom of the bath. The strips were set up with an initial tension of 0.1–0.3 g and mechanical activity was recorded using a pen-recorder.

For intracellular recording, a conventional microelectrode filled with 3 M KCl (30–50 M $\Omega$ ) was inserted from the outer surface of the preparation. The chamber in which the muscle preparation was mounted had a volume of 2 ml, and was perfused at a rate of 3 ml min<sup>-1</sup> and at a temperature of 35–36°C.

The double sucrose gap method was used to record both membrane potential and tension of the tissue. The chamber used has been described in detail elsewhere (Ito & Tajima, 1981a). Neurogenic responses were produced by field stimulation with a ring electrode placed in the centre pool of the apparatus. The current pulse used was 50–100  $\mu\text{s}$  in duration and about 10–30 V in strength. Drugs were dissolved in Krebs solution and exposed to the tissue through the central pool of the double sucrose gap apparatus, using a multi-way tap (dead-time approximately 15 s).

The following drugs were used; procaterol ((±)-procaterol, Otsuka Pharmac. Co.), isoprenaline ((-)-isoprenaline (+)-bitartrate, Nakai Chemicals), and ICI 118551 erythro-DL-1(7-methylindan-4-yloxy)-3-isopropylamin)butan-2-ol (a  $\beta_2$ -adrenoceptor blocker, ICI Pharmaceuticals.), indomethacin and acetylcholine hydrochloride (Sigma) and guanethidine (Tokyo Kasei).

Results (amplitude of contractions and e.j.ps) are expressed as mean  $\pm$  s.d. and were analysed for statistical significance by Student's *t* test.

## Results

### *Effects of procaterol and isoprenaline on twitch contractions of dog trachea evoked by field stimulation*

The effects of procaterol on twitch contractions evoked by field stimulation of cholinergic nerve

fibres were studied in dog tracheal tissue. The dog airway smooth muscle cells are innervated by both cholinergic and adrenergic nervous systems (Russell, 1980; Ito & Tajima, 1981a) and noradrenaline released from sympathetic nerve terminals can activate pre-junctional  $\beta$ -adrenoceptors to suppress cholinergic transmission (Danser *et al.*, 1987). Also twitch contractions evoked by nerve stimulation decreased progressively in amplitude because endogenous prostaglandin E compounds inhibit transmitter release from the vagus nerve terminals (Ito & Tajima, 1981a,b; Inoue *et al.*, 1984; Walters *et al.*, 1984; Inoue & Ito, 1986; Shore *et al.*, 1987). Therefore the experiments were carried out in the combined presence of guanethidine ( $10^{-6}$  M) and indomethacin ( $10^{-5}$  M).

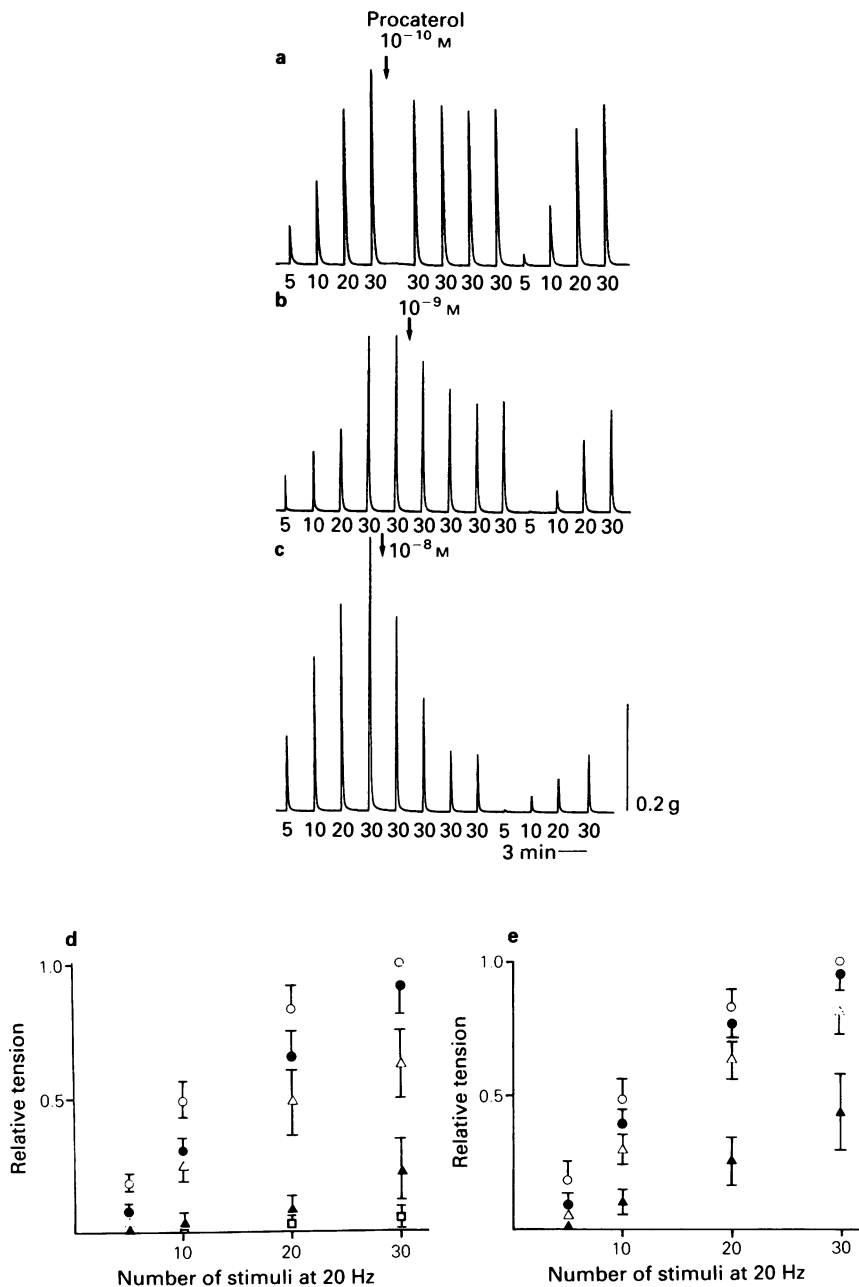
Figure 1a–c shows the effects of procaterol ( $10^{-10}$ ,  $10^{-9}$  and  $10^{-8}$  M) on the amplitude of twitch contractions evoked by repetitive field stimulation (5, 10, 20 and 30 stimuli at 20 Hz) applied every 3 min. Procaterol ( $10^{-10}$  M) had no effects on the resting tension but did suppress the amplitude of twitch contractions (to 0.25–0.7 times the control value) when fewer than 20 stimuli were applied (see also Figure 1d). With increased concentrations ( $10^{-9}$ – $10^{-8}$  M), this drug dose-dependently suppressed the amplitude of the twitch contractions evoked by nerve stimulations at 20 Hz. The results are summarized in Figure 1d.

For comparison, the effects of isoprenaline on twitch contractions were also observed. Isoprenaline, similarly produced a dose-dependent suppression of the amplitude of twitch contractions, although higher concentrations ( $10^{-9}$ – $10^{-7}$  M) than those of procaterol were required (Figure 1e).

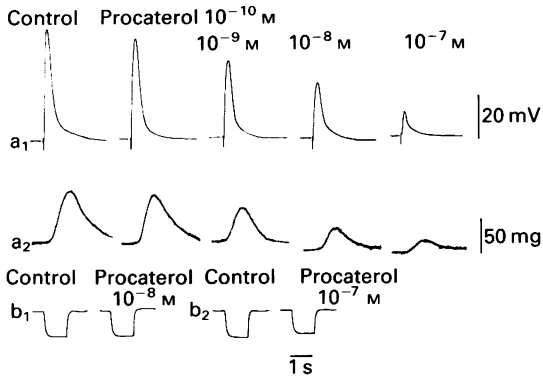
### *Effects of procaterol and isoprenaline on the amplitude of the excitatory junction potential (e.j.p.)*

The mechanisms involved in the inhibitory effects of procaterol on twitch contractions were investigated with the double sucrose gap method in the presence of guanethidine and indomethacin.

With the double sucrose gap technique, field stimulation evoked an excitatory junction potential (e.j.p.) and contraction (Figure 2). Procaterol ( $10^{-10}$  and  $10^{-9}$  M), significantly suppressed the e.j.p. amplitude to respectively  $0.92 \pm 0.03$  times ( $n = 7$ ,  $P < 0.01$ ) and to  $0.70 \pm 0.08$  times the control ( $n = 8$ ,  $P < 0.01$ ) when a single stimulus was applied with no change in the membrane potential and input membrane resistance. In parallel to the reduction in e.j.p. amplitude, the amplitude of the twitch contraction was also suppressed. At higher concentrations ( $10^{-8}$ – $10^{-7}$  M) procaterol reduced the resting tension and further suppressed the amplitude of the e.j.p. and the twitch contractions in a dose-dependent manner.



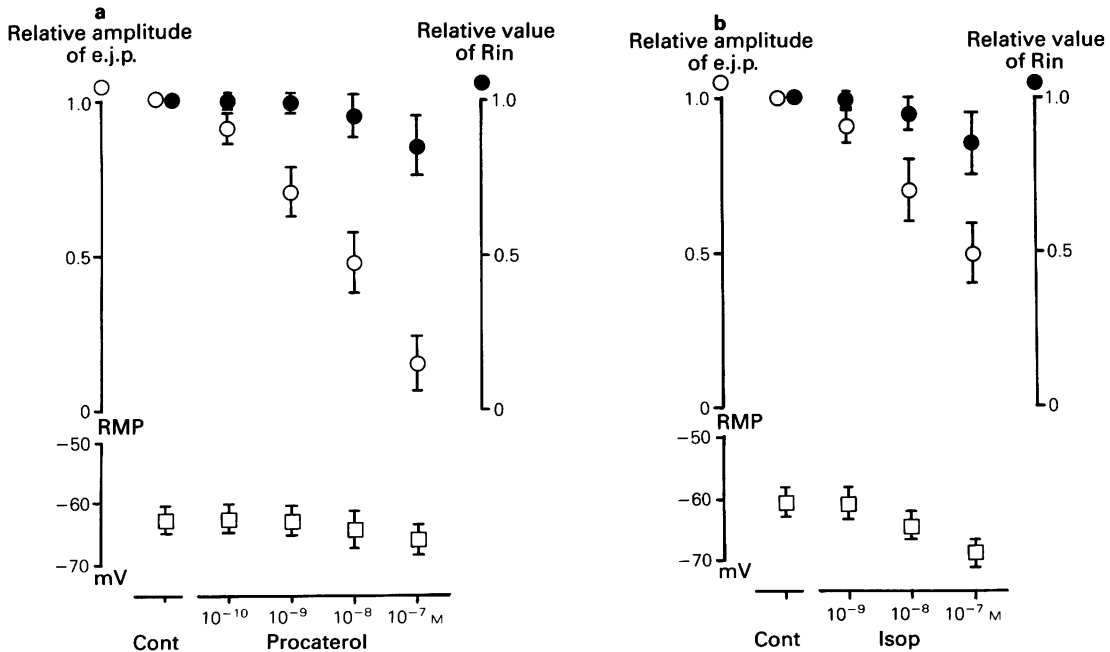
**Figure 1** Effects of various concentrations of procatenol ( $10^{-10}$ – $10^{-7}$  M) and isoprenaline ( $10^{-9}$ – $10^{-7}$  M) on twitch contractions evoked by field stimulation (5, 10, 20 and 30 stimuli at 20 Hz). Arrows indicate application of the agents. (a, b and c) Effects of procatenol ( $10^{-10}$ – $10^{-8}$  M) on the twitch amplitude; (d and e) relationship between the number of stimuli (5–30 at 20 Hz) and relative amplitude of twitch contractions in the presence or absence of procatenol (d) and isoprenaline (e). The amplitude of twitch contractions evoked by 30 stimuli at 20 Hz in normal Krebs solution was defined as relative tension of 1.0. In (d): (○) control; procatenol  $10^{-10}$  M (●),  $10^{-9}$  M (△),  $10^{-8}$  M (▲) and  $10^{-7}$  M (□). In (e): (○) control; isoprenaline  $10^{-9}$  M (●);  $10^{-8}$  M (△) and  $10^{-7}$  M (▲).



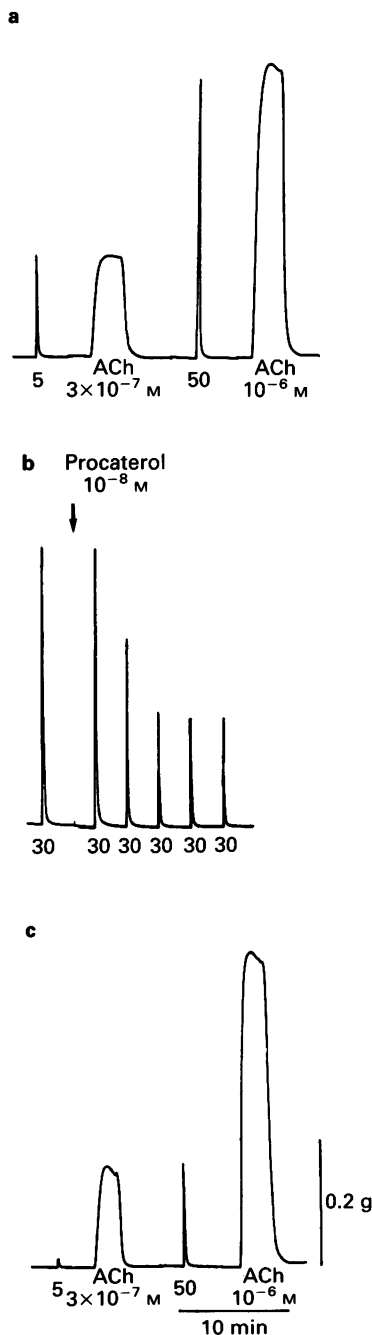
**Figure 2** Effects of various concentrations of procaterol on the amplitude of e.j.p.s ( $a_1$ ) and twitch contractions ( $a_2$ ) evoked by a single pulse with field stimulation. Sucrose gap recording of membrane potential and tension. ( $b_1$  and  $b_2$ ) Effects of procaterol  $10^{-8}$  or  $10^{-7}$  M on the amplitude of electrotonic potentials of the smooth muscle cells evoked by constant square pulses ( $5 \times 10^{-7}$  A).

Figure 2b shows effects of procaterol ( $10^{-8}$  or  $10^{-7}$  M) on the electrotonic potentials evoked by constant inward currents ( $5 \times 10^{-7}$  A). Procaterol  $10^{-7}$  M reduced the amplitude of the electrotonic potential. The input membrane resistance, measured from the change in the amplitude of electrotonic potential, was reduced to  $0.85 \pm 0.09$  ( $\pm$  s.d.,  $n = 7$ ) of the control value.

Figure 3(a and b) summarizes the effects of procaterol and isoprenaline on the relative amplitude of the e.j.p., the input membrane resistance and resting membrane potential of the smooth muscle cells. Low concentrations of procaterol ( $10^{-10}$ – $10^{-8}$  M) suppressed the e.j.p. amplitude with no change in the input membrane resistance and resting membrane potential of the smooth muscle cells. At  $10^{-7}$  M, however, this drug significantly hyperpolarized the membrane and reduced the input membrane resistance. Isoprenaline ( $10^{-9}$  and  $10^{-8}$  M), similarly, suppressed the e.j.p. amplitude without any change in the input membrane resistance or resting membrane potential.



**Figure 3** Relationship between the concentration of procaterol (a) and isoprenaline (b) and the relative amplitude of the e.j.p. (○), relative value of input membrane resistance ( $R_{in}$ ) (●) and the resting membrane potential (RMP) (□) of the smooth muscle cells. The amplitude of e.j.p. evoked by single field stimulation in normal Krebs solution, and the amplitude of electrotonic potentials produced by square pulses in normal Krebs solution was defined as 1.0. Each point is the mean value derived from 5–20 experiments; vertical bars indicate  $2 \times$  s.d.. Sucrose gap recording of e.j.p. and electrotonic potentials was used, and resting membrane potential was recorded by microelectrode recording.



**Figure 4** Effects of procatерол ( $10^{-8}$  M) on the amplitude of twitch contraction evoked by field stimulation (5 or 50 stimuli at 20 Hz) and by acetylcholine (ACh,  $3 \times 10^{-7}$  or  $10^{-6}$  M). (a) Twitches (evoked by 5 and 50 stimuli at 20 Hz) and ACh-induced contractions ( $3 \times 10^{-7}$  and  $10^{-6}$  M) in normal Krebs solution (a), and in the presence of procatерол ( $10^{-8}$  M, b and c).

#### *Effects of procatерол on the acetylcholine (ACh)-induced contractions of the dog trachea*

The effects of procatерол on the response to ACh were examined in order to determine the site of action of procatерол.

As shown in Figure 4, the amplitudes of the ACh ( $3 \times 10^{-7}$  or  $10^{-6}$  M)-induced contractions were comparable to that of twitches evoked by 5 or 50 stimuli at 20 Hz. Application of procatерол ( $10^{-8}$  M) decreased the twitch contraction amplitudes to 5 or 50 stimuli to respectively  $0.05 \pm 0.05$  times ( $n = 3$ ) and  $0.41 \pm 0.10$  ( $n = 3$ ) the control values. However, the amplitude of the ACh-induced contractions was unaffected which indicates that procatерол had no effect on the ACh-sensitivity of the smooth muscle cells.

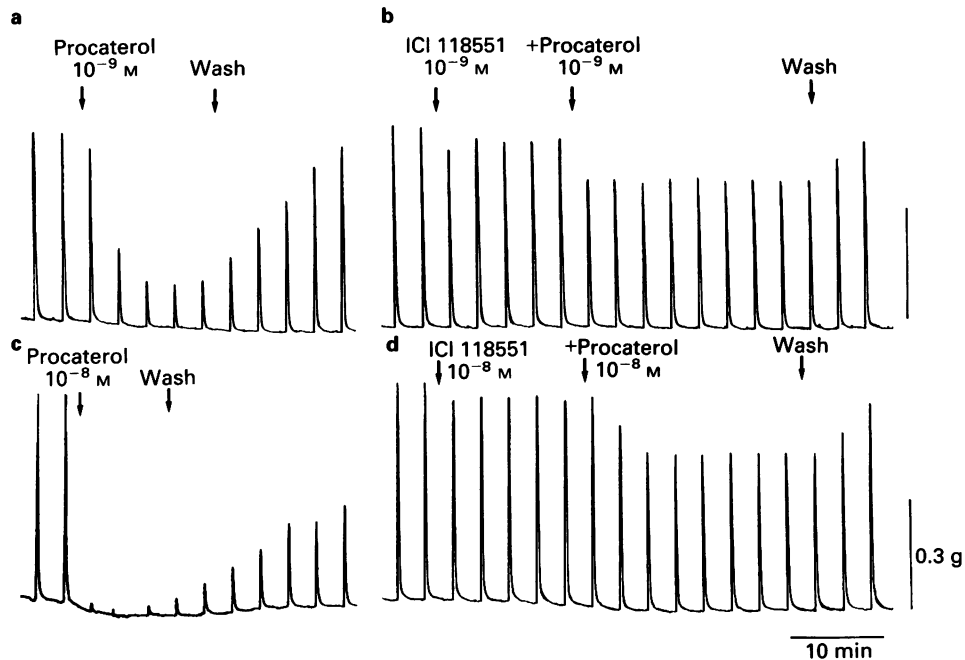
#### *Effects of ICI-118551, a selective $\beta_2$ -adrenoceptor blocking agent, on the action of procatерол*

The effects of ICI-118551, a  $\beta_2$ -adrenoceptor blocking agent (Bilski *et al.*, 1983), on the action of procatерол in the presence of guanethidine ( $10^{-6}$  M) and indomethacin ( $10^{-5}$  M) were observed in order to clarify the subtype of  $\beta_2$ -adrenoceptors in the vagus nerve terminal. The  $K_d$  values of ICI-118551 against  $\beta_2$ -adrenoceptor measured in guinea-pig trachea, bronchi and parenchyma ranged between  $10^{-9}$ – $7 \times 10^{-9}$  M (Carswell & Nakorski, 1983).

Figure 5 shows the effects of pretreatment with ICI-118551 ( $10^{-9}$  or  $10^{-8}$  M) on the action of procatерол ( $10^{-9}$  or  $10^{-8}$  M). In the absence of ICI-118551,  $10^{-9}$  and  $10^{-8}$  M procatерол reduced the twitch amplitude (5 stimuli at 20 Hz) to respectively  $0.25 \pm 0.08$  and to  $0.07 \pm 0.03$  of the control values ( $n = 7$ ). Application of  $10^{-9}$  and  $10^{-8}$  M ICI-118551 itself slightly reduced the twitch amplitude (to  $0.95 \pm 0.05$  and to  $0.87 \pm 0.07$  of the control value,  $n = 5$  and 4 respectively) and suppressed the action of procatерол ( $10^{-9}$  or  $10^{-8}$  M) on the twitch amplitude. Thus procatерол suppressed the twitch amplitude to  $0.78 \pm 0.09$  ( $n = 3$ ) or to  $0.72 \pm 0.05$  ( $n = 3$ ) of the control value in the presence of  $10^{-9}$  and  $10^{-8}$  M ICI-118551 respectively. These results indicate that the inhibitory effects of procatерол ( $10^{-9}$  or  $10^{-8}$  M) on the twitch amplitude are mainly due to activation of  $\beta_2$ -adrenoceptors.

#### **Discussion**

The results presented here clearly show that procatерол acts prejunctionally to inhibit excitatory neuro-effector transmission, presumably by suppressing acetylcholine release from the vagus nerve terminals in the dog trachea, in addition to the



**Figure 5** Effects of pretreatment of the tissue with ICI-118551 ( $10^{-9}$  and  $10^{-8}$  M) on the action of procaterol ( $10^{-9}$  and  $10^{-8}$  M) on contractions evoked by field stimulation (5 stimuli at 20 Hz). Effects of procaterol ( $10^{-9}$  and  $10^{-8}$  M) were observed before (a and c) and after the pretreatment with ICI-118551 ( $10^{-9}$  and  $10^{-8}$  M) (b and d).

well-documented direct actions on the airway smooth muscle cells (Yoshizaki *et al.*, 1976; Yabuuchi *et al.*, 1977; Himori & Taira, 1977; Tei *et al.*, 1979; Yamashita *et al.*, 1987; Fujiwara *et al.*, 1988). Low concentrations of procaterol ( $10^{-10}$ – $10^{-9}$  M) significantly suppressed the amplitude of e.j.p. and contractions evoked by nerve stimulation without changing the resting membrane potential, input membrane resistance or ACh-sensitivity of the smooth muscle cells. The results seen with isoprenaline on twitch and e.j.p. amplitude are in agreement with earlier work and confirmed the presence of inhibitory prejunctional  $\beta$ -adrenoceptors on the vagus nerve terminals in the dog trachea (Ito & Tajima, 1982).

In anaesthetized dogs and guinea-pigs, procaterol, given intravenously, was almost equiactive with isoprenaline in inhibiting the increase in bronchial resistance induced by histamine (Yabuuchi *et al.*, 1977; Tei *et al.*, 1977). However, in conscious guinea-pigs procaterol given orally or by aerosol, was about 2 and 8 times more potent respectively, than isoprenaline in prolonging the time to severe dyspnoea after exposure to histamine aerosol (Tei *et al.*, 1977; Yabuuchi, 1977).

In contrast, in isolated tracheal tissue procaterol and isoprenaline were equipotent in suppressing the

mechanical responses evoked by acetylcholine, histamine or 5-hydroxytryptamine. Further, both agents had much the same effects on the levels of cyclic AMP in the smooth muscle cells in dog trachea. These chemicals in concentrations over 1 nM, increased the amount of cyclic AMP in a dose-dependent manner and relaxed the tissue to the same extent. Procaterol and isoprenaline were equipotent in suppressing the intracellular free  $\text{Ca}^{2+}$  concentration in smooth muscle cells evoked by various agents, as estimated from fura-2 fluorescence (Fujiwara *et al.*, 1988). In the present experiments, procaterol was roughly ten times more potent than isoprenaline in suppressing the amplitude of e.j.p. and twitch contractions. Assuming that procaterol and isoprenaline are equipotent in suppressing the mechanical responses of the isolated smooth muscle cells of dog trachea (Fujiwara *et al.*, 1988), the pre-junctional action of procaterol observed in the present results may explain why this drug is more potent than isoprenaline, in conscious animals (Tei *et al.*, 1977; Yabuuchi, 1977).

It was recently reported that noradrenaline when released from sympathetic nerve endings can activate pre-junctional inhibitory  $\beta_1$ -adrenoceptors to depress cholinergic neurotransmission in the dog bronchial wall (Danser *et al.*, 1987). In contrast,

in the present experiments, procaterol, a  $\beta_2$ -adrenoceptor agonist, suppressed the amplitude of e.j.p. and twitch contractions to a greater extent than did isoprenaline, and this effect was suppressed by a selective  $\beta_2$ -adrenoceptor antagonist ICI-118551. These results raise the possibility that  $\beta_2$ -adrenoceptors exist on the vagus nerve terminals, in addition to  $\beta_1$ -adrenoceptors.

## References

- BILSKI, A.J., HALLIDAY, S.E., FITZGERALD, J.D. & WALE, J.L. (1983). The pharmacology of a  $\beta_2$ -selective adrenoceptor antagonist (ICI 118551). *J. Cardiovasc. Pharmacol.*, **5**, 430–437.
- BORGEN, L.A. (1987). A six-month multicenter trial of terbutaline and procaterol. *J. Respir. Dis.*, Supplement S58–S60.
- CARSWELL, H. & NAHORSKI, S.R. (1983).  $\beta$ -Adrenoceptor heterogeneity in guinea-pig airways: comparison of functional and receptor labelling studies. *Br. J. Pharmacol.*, **79**, 965–971.
- DANSER, A.H.J., VAN DEN ENDE, R., LOREUZ, R.R., FLAVAHAN, N.A. & VANHOUTTE, P.M. (1987). Prejunctional  $\beta_1$ -adrenoceptors inhibit cholinergic transmission in canine bronchi. *J. Appl. Physiol.*, **62**, 785–790.
- DELHAYE, M., TATON, G., CAMUS, J.C., CHATELAIN, P., ROBBERECHT, P., WAELBROECK, M. & CHRISTOPHE, J. (1983). Effects of full and partial  $\beta$ -adrenergic agonists and antagonists on human lung adenylate cyclase. *Biochem. Pharmacol.*, **32**, 1831–1835.
- FUJIWARA, T., SUMIMOTO, K., ITOH, T., SUZUKI, H. & KURIYAMA, H. (1988). Relaxing actions of procaterol, a  $\beta_2$ -adrenoceptor stimulant, on smooth muscle cells of the dog trachea. *Br. J. Pharmacol.*, **93**, 199–209.
- HIMORI, N. & TAIRA, N. (1977). Assessment of the selectivity of OPC-2009, a new  $\beta_2$ -adrenoceptor stimulant, by the use of the blood-perfused trachea in situ and of the isolated and blood-perfused papillary muscle of the dog. *Br. J. Pharmacol.*, **61**, 9–17.
- INOUE, T., ITO, Y. AND TAKEDA, K. (1984). Prostaglandin-induced inhibition of acetylcholine release from neuronal elements of dog tracheal tissue. *J. Physiol.*, **349**, 553–570.
- INOUE, T. & ITO, Y. (1986). Characteristics of neuro-effector transmission in the smooth muscle layer of dog bronchiole and modifications by autacoids. *J. Physiol.*, **370**, 551–565.
- 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. Pharmacol.*, **73**, 563–571.
- The potent bronchodilator effects of procaterol observed in clinical use (Borgen, 1987) may be explained by the dual action of this agent on the pre- and post-junctional  $\beta_2$ -adrenoceptors in the airway smooth muscle tissues.
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- ITO, Y. & TAJIMA, K. (1982). Dual effects of catecholamines on pre- and post-junctional membranes in the dog trachea. *Br. J. Pharmacol.*, **75**, 433–440.
- RUSSELL, J.A. (1980). Noradrenergic inhibitory innervation of canine airways. *J. Appl. Physiol.*, **48**, 16–22.
- SHORE, S., COLLIER, B. & MORTIN, J.G. (1987). Effects of endogenous prostaglandins on acetylcholine release from dog trachealis muscle. *J. Appl. Physiol.*, **62**, 1837–1844.
- TAKAYANAGI, I., KONDO, N., YAMASHITA, H., HONGO, T. & TAKAGI, K. (1977). New selective  $\beta_2$ -adrenoceptor stimulants 5-(1-hydroxy-2-ethylaminobutyl)-8-hydroxy carbostyryl hydrochlorides (OPC-2009 and OPC-2030) and cyclic AMP concentration. *J. Pharmac. Pharmacol.*, **29**, 187–189.
- TEI, S., YAMASHITA, S. & YABUUCHI, Y. (1979). The  $\beta$ -adrenoceptor stimulant action of procaterol, a new  $\beta_2$ -adrenoceptor stimulant, on bronchial resistance and heart rate in guinea-pigs. *Pharmacometrics*, **17**, 335–343.
- WALTERS, E.H., O'BYRNE, P.M., FABBIR, L.M., GOF, P.D., HOLTZMAN, M.J. & NADEL, J.A. (1984). Control of neurotransmission by prostaglandins in canine trachealis smooth muscle. *J. Appl. Physiol.*, **57**, 129–134.
- YABUUCHI, Y. (1977). The  $\beta$ -adrenoceptor stimulant properties of OPC-2009 on guinea-pig isolated tracheal, right atrial and left atrial preparations. *Br. J. Pharmacol.*, **61**, 513–521.
- YABUUCHI, Y., YAMASHITA, S. & TEI, S. (1977). Pharmacological studies of OPC-2009, a newly synthesized selective beta adrenoceptor stimulant, in the bronchomotor and cardiovascular system of the anesthetized dog. *J. Pharmacol. Exp. Ther.*, **202**, 326–336.
- YAMASHITA, S., TAKAI, M. & YABUUCHI, Y. (1978). Actions of procaterol (OPC-2009), a new  $\beta_2$ -adrenoceptor stimulant, on pulmonary resistance, contractions of the soleus muscle, and cardiovascular system of the anesthetized cat. *J. Pharmac. Pharmacol.*, **30**, 273–279.
- YOSHIZAKI, S., TANIMURA, K., TAMADA, S., YABUUCHI, Y. & NAKAGAWA, K. (1976). Sympathomimetic amines having a carbostyryl nucleus. *J. Med. Chem.*, **19**, 1138–1142.

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