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Effects of vasoactive intestinal polypeptide antagonists on cholinergic neurotransmission in dog and cat trachea

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1 The effects of vasoactive intestinal polypeptide (VIP) antagonists [AC-Tyr[1], D-Phe[2]]-GRF(1-29)-NH_2 and [4-Cl-D-Phe[6], Leu[17]]-VIP on excitatory neuroeffector transmission in the dog and cat trachea were investigated by use of microelectrode, double sucrose-gap and tension recording methods.

2 In the dog trachea, repetitive stimuli at high frequency (20 Hz) markedly enhanced the amplitude of contraction, the amplitude of contractions evoked by 50 stimuli at 20 Hz relative to that evoked by 5 stimuli being 14.2 ± 3.8 times ($n = 7$, ± s.d.). In the cat, the summation was much less marked, the amplitude of contractions evoked by 50 stimuli relative to that evoked by 5 stimuli being only 2.1 ± 0.6 times ($n = 5$, ± s.d.). Neither VIP antagonist had any effect on the relationship between the number of stimuli at 20 Hz and the relative amplitude of contraction in the dog trachea, but did enhance the amplitude of contractions to 1.1–1.5 times control in the cat trachea.

3 VIP antagonists dose-dependently enhanced the amplitude of excitatory junction potentials (e.j.ps) evoked by a single stimulus in the cat trachea, without changing the resting membrane potential or input membrane resistance of the smooth muscle cells. However, neither antagonist had any effect on the amplitude of the e.j.p. in the dog trachea.

4 Neither VIP antagonist had any effect on the post-junctional response of smooth muscle cells to exogenously applied acetylcholine (ACh; 10^{-9}–10^{-5} M) in the dog or cat trachea.

5 In the cat trachea, VIP (10^{-11} M) suppressed the e.j.p. amplitude to 0.74 ± 0.09 times the control value ($n = 6$). However, after pretreatment of the tissue with the VIP antagonists [Ac-Tyr[1], D-Phe[2]]-GRF(1-29)-NH_2 (10^{-8} M) and [4-Cl-D-Phe[6], Leu[17]]-VIP (10^{-8} M), VIP (10^{-11} M) did not suppress the e.j.p. amplitude, indicating that VIP antagonists block the presynaptic inhibitory action of exogenous VIP.

6 In parallel with the enhancement of contraction, e.j.ps showed marked summation when repetitive field stimulations were applied at high frequency (20 Hz) in the dog trachea. The relationship between the relative amplitude of the e.j.p. and number of stimuli at 20 Hz was linear and the slope was 2.2 ± 0.3 mV/stimulation. VIP antagonists did not affect this relationship. However, in the cat trachea, summation of e.j.ps was not at all marked and a linear relationship was not observed with the double sucrose-gap method. Incubation of the cat tracheal tissue with either of the VIP antagonists (10^{-8} or 10^{-7} M) markedly enhanced the summation of e.j.ps evoked by repetitive field stimulation at 20 Hz, and after the treatment a linear relationship between the number of stimuli and the amplitude of e.j.ps was observed, the slopes being 0.6 ± 0.1 ($n = 8$) and 0.55 ± 0.1 mV/stimulation ($n = 5$), respectively.

7 These results indicate that both VIP antagonists, [Ac-Tyr[1], D-Phe[2]]-GRF(1-29)-NH_2 and [4-Cl-D-Phe[6], Leu[17]]-VIP, have a prejunctional action accelerating the excitatory neuroeffector transmission, presumably by enhancing transmitter release from the vagus nerves in the cat, but not in the dog trachea.

Keywords: VIP; VIP-antagonists; trachea; cholinergic neurotransmission; modulation; excitatory junction potentials (e.j.ps)

Introduction

Recent investigations have revealed that the parasympathetic nervous supply to the airways contains non-adrenergic non-cholinergic (NANC) inhibitory neurotransmitters such as vasoactive intestinal polypeptide (VIP), peptide histidine isoleucine (PHI) or peptide histidine methionine (PHM) in addition to acetylcholine (see, for example, Barnes, 1986). The presence of NANC inhibitory neurotransmitters in the nervous system was first demonstrated by relaxation of the airway smooth muscle produced in response to electrical field stimulation in the presence of cholinergic and adrenergic blocking agents in the guinea-pig (Coburn & Tomita, 1973), baboon (Middendorf & Russell, 1978), cat (Ito & Takeda, 1982) and human (Richardson & Be'land, 1976). Subsequently, immunofluorescence techniques demonstrated the presence of VIP-immunoreactive nerve fibres in human and animal airway smooth muscle layers including those of the dog and cat (Dey *et al.*, 1981; Polak & Bloom, 1982; Hakanson *et al.*, 1983). Further, VIP-immunoreactivity was localized to cholinergic nerves (Lundberg, 1981; Laitinen *et al.*, 1985a), and ultrastructural studies have demonstrated the presence of peptide containing granules within cholinergic nerves in the airway smooth muscle (Laitinen *et al.*, 1985b).

Hakoda & Ito (1990) reported that VIP, exogenously applied in low concentrations, has a prejunctional action which inhibits excitatory neuroeffector transmission in the dog and cat trachealis, and that commercially available VIP antiserum or the VIP antagonists [Ac-Tyr[1], D-Phe[2]]-GRF(1-29)-NH_2 and [4-Cl-D-Phe[6], Leu[17]]-VIP enhance the amplitude and summation of excitatory junction potentials (e.j.ps) in the trachea of the cat, but not of the dog. Further, overnight incubation of the cat tracheal tissue with VIP antiserum markedly reduced the amplitude of the muscle relaxation evoked by electrical field stimulation during contraction induced by 5-hydroxytryptamine in the presence of atropine (10^{-5} M) and guanethidine (10^{-6} M). These observations indicate that in the cat trachea, VIP may contribute to NANC relaxation and that endogenous VIP also inhibits the release of ACh from the vagus nerve when a single stimulus or repetitive stimuli are applied (Hakoda & Ito, 1990).

In the dog, on the other hand, although the occurrence of VIP-immunoreactive nerves in the walls of bronchi, bronchioles and pulmonary vessels has been demonstrated (Dey *et al.*, 1981), it has been reported that NANC relaxations are not demonstrable in tracheal or bronchiolar tissues (Ito & Tajima,

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1981; Inoue & Ito, 1986). In previous work, the actions of VIP-antagonists on dog tracheal tissue were not investigated systematically. To study the possible role of endogenous VIP on excitatory neuro-effector transmission in the airways, we have performed comparative studies on the actions of the VIP antagonists [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 and [4-Cl-D-Phe^6, Leu^{17}]-VIP on excitatory neuro-effector transmission in dog and cat trachea.

Methods

Adult mongrel dogs and cats of either sex, weighing 10–13 kg and 2–4 kg respectively were anaesthetized with i.v. administration of pentobarbitone (30 mg kg^{-1}). Segments of cervical trachea were excised, and a dorsal strip of transversely running smooth muscle was separated from the cartilage. The mucosa and adventitial areolar tissue were carefully removed. The tracheal smooth muscle was cut into a strip 2.0–2.5 mm wide and about 15 mm long for the double sucrose gap method. 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 bubbled with 97% O_2 and 3% CO_2 and the pH was 7.3–7.4. The double-sucrose gap method was used to record the membrane potential and tension development in the tissue. The chamber used has been described elsewhere (Ito & Tajima, 1981). To produce neurogenic responses, electrical field stimulation was applied via a ring electrode placed in the centre pool of the apparatus using an electronic stimulator (Nihon Kohden SEN-7103). Single and repetitive stimulation was applied with current pulses of 50 μs duration and about 10–20 V intensity. The voltage of the current pulse was adjusted so that an e.j.p. of a certain fixed amplitude was evoked by a single pulse. Drugs were dissolved in Krebs solution and applied to the tissue through the centre pool of the double sucrose-gap apparatus with a multi-way tap (dead-time approximately 30 s).

For intracellular recording of the membrane potential from single cells, thin strips of tissue 10–15 mm long, 4–5 mm wide and 0.3–0.4 mm thick were used. A conventional microelectrode filled with 3 M KCl (30–50 MΩ) was inserted from the outer surface of the preparation. Field stimulation was applied to the nerves through a pair of Ag-AgCl wires 3–5 mm apart, placed so that a current pulse would pass transversely across the tissue. Single and repetitive stimuli at 20 Hz were applied, with a pulse of 30–100 μs duration and 30–50 V intensity, using an electronic stimulator (Nihon Kohden SEN-7103). The chamber in which the strips were mounted had a volume of 2 ml, and was superfused at a rate of 3 ml min^{-1} at a temperature of 35–36°C. To avoid recording artifacts due to twitch-like contractions of the muscle tissue, the preparation was firmly fixed onto the rubber plate in the chamber with insect pins of 100 μm diameter.

For the measurement of the mechanical changes, the tissue was mounted in a 1 ml organ bath through which the test solution flowed continuously at a rate of 3 ml min^{-1} at a temperature of 35–36°C. The preparation was placed vertically and one end of each strip was tied to a mechanotransducer (Nihon-Kohden Ltd., RCA-5734) and the other end to a hook at the bottom of the bath with fine silk thread. The strips were set up with an initial tension of 0.1–0.2 g and mechanical activity was recorded with a pen recorder.

The following drugs were used: indomethacin and acetylcholine hydrochloride (Sigma), guanethidine (Tokyo kasei), vasoactive intestinal polypeptide (VIP) (Peptide Institute, Osaka, Japan), VIP antagonists, [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 and [4-Cl-D-Phe^6, Leu^{17}]-VIP (Cosmo Bio. Co. Ltd., Tokyo) and atropine sulphate (Daiichi).

Results (amplitude of contractions or e.j.ps) are expressed as mean ± s.d. and were analyzed for statistical significance with Student's t test.

Results

Effects of VIP antagonists [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 and [4-Cl-D-Phe^6, Leu^{17}]-VIP on contraction of dog and cat trachea evoked by electrical field stimulation

The effects of VIP antagonists on the contractions evoked by electrical field stimulation of cholinergic nerve fibres were studied in dog and cat tracheal tissues. The dog and cat airway smooth muscle cells are innervated by both cholinergic and adrenergic nerves (Russell, 1980), and noradrenaline released from sympathetic nerves can activate prejunctional β-adrenoceptors to inhibit cholinergic neurotransmission. In the dog trachea, the contraction evoked by nerve stimulation decreased progressively in amplitude, because endogenous prostaglandin E compounds inhibit cholinergic transmitter release from the vagus nerves (Ito, 1990). Therefore, the experiments on the dog trachea were carried out in the combined presence of guanethidine (10^{-6} M) and indomethacin (10^{-5} M), but those on the cat trachea in the presence of guanethidine (10^{-6} M) alone.

Figure 1 shows the effects of the VIP antagonist [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 on the amplitude of contractions evoked by repetitive field stimulation (5, 10, 20, 30 and 50 stimuli at 20 Hz) in trachea of the dog (a and a') and cat (b and b'). One of most striking differences between the dog and cat trachea was that the contraction was monophasic in the dog (Ito & Tajima, 1981) but biphasic in the cat trachea (Ito & Takeda, 1982). There was also a pronounced difference in the summation of the amplitude of contraction. Namely, in the dog trachea, repetitive stimuli at high frequency (20 Hz) markedly enhanced the amplitude of the contraction, the amplitude of contraction evoked by 50 stimuli at 20 Hz being 14.2 ± 3.8 times (n = 7, range 10–16 times) that evoked by 5 stimuli. On the other hand, in the cat trachea, repetitive stimuli at 20 Hz enhanced the amplitude of the twitch contraction only slightly, the amplitude of contraction evoked by 50 stimuli at 20 Hz being 2.1 ± 0.5 times (n = 5, range 1.7–2.5 times) that evoked by 5 stimuli.

A VIP antagonist, [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 (10^{-7} M), changed neither the resting tone of the muscle preparation nor the amplitude of twitch contraction evoked by repetitive stimuli in the dog trachea (Figure 1a and a'). On the other hand, in the cat trachea, the same antagonist (> 10^{-8} M) significantly increased the amplitude of contractions evoked by repetitive stimuli at 20 Hz (Figure 1b and b'). Similar results were obtained with the other VIP antagonist [4-Cl-D-Phe^6,Leu^{17}]-VIP (> 10^{-7} M): this too significantly increased the amplitude of contraction in the trachea of the cat (Figure 1e and f), but not of the dog (Figure 1c and d).

Effects of VIP antagonists on the amplitude of excitatory junction potentials (e.j.ps)

The mechanisms involved in the enhancement by VIP antagonists of twitch contractions in the cat trachea were studied and the effects were compared with those in the dog trachea by use of microelectrode and double sucrose gap-methods. A single field stimulation evoked an e.j.p. and the VIP antagonist, [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 (10^{-9}–10^{-7} M) produced no effects on either the e.j.ps or the resting membrane potential of the smooth muscle cells measured by the microelectrode in the dog trachea. The other VIP antagonist [4-Cl-D-Phe^6, Leu^{17}]-VIP (10^{-9} M) also produced no effects on the amplitude of e.j.ps. However, at increased concentration (10^{-7} M), this antagonist suppressed the e.j.p. amplitude to 0.81 ± 0.05 times control (n = 5, P < 0.01) without changing the resting membrane potential in the dog trachea (Figure 2a and b).

In the cat trachea, both VIP antagonists enhanced the amplitude of e.j.ps. For example, 10^{-10} M [Ac-Tyr^1, D-Phe^2]-

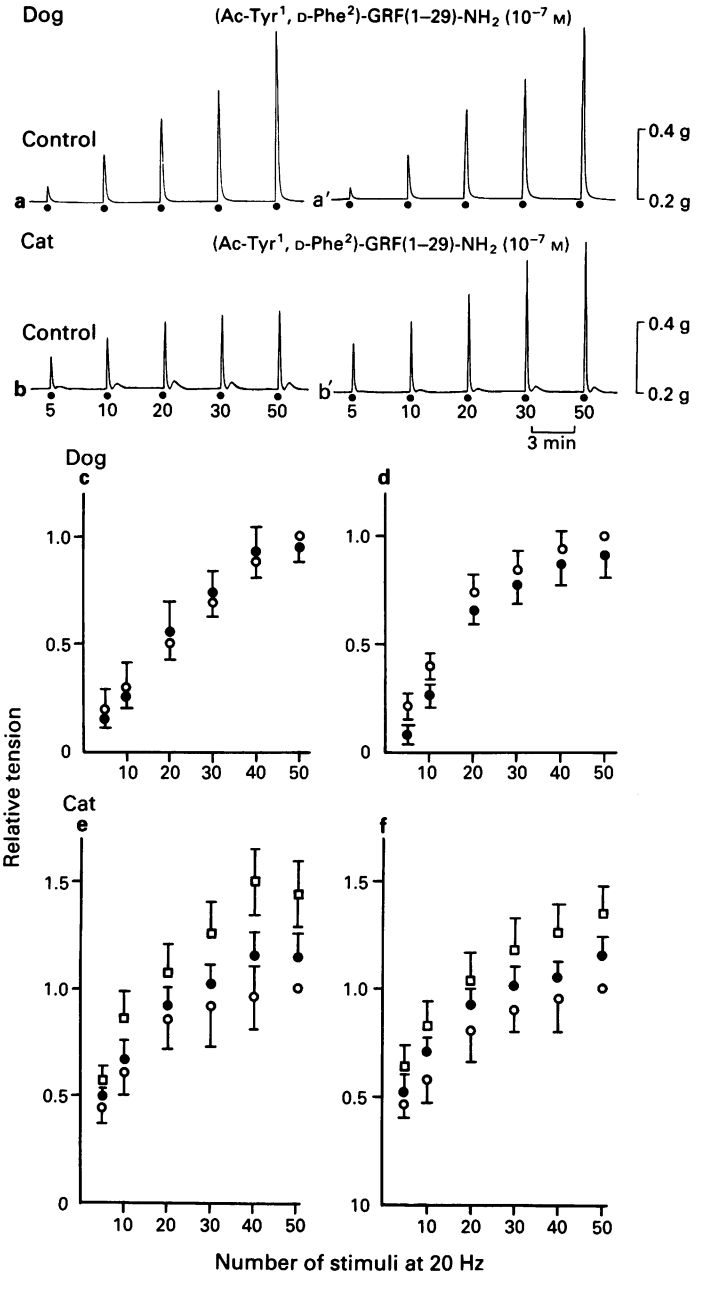

Figure 1 Effects of the vasoactive intestinal polypeptide (VIP) antagonist [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 on twitch contractions of dog (a and a') and cat (b and b') trachea evoked by field stimulation (5, 10, 20, 30 and 50 stimuli at 20 Hz). Effects of VIP antagonists [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 (c and e) and [4-Cl-D-Phe^6, Leu^{17}]-VIP (d and f) on the relationship between the number of stimuli (5–50 at 20 Hz) and relative amplitude of twitch contractions in the dog and cat trachea. In (c–f): (○) control; [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 10^{-7} M (●) in (c), 10^{-9} M (●) and 10^{-8} M (□) in (e); [4-Cl-D-Phe^6, Leu^{17}]-VIP 10^{-7} M (●) in (d), 10^{-8} M (●) and 10^{-7} M (□) in (f). The amplitude of twitch contractions evoked by 50 stimuli at 20 Hz in normal Krebs solution was given the relative value 1.0.

GRF(1-29)-NH_2 increased the e.j.p. amplitude to 1.21 ± 0.06 times control ($n = 6$, $P < 0.01$), and 10^{-8} M [4-Cl-D-Phe^6, Leu^{17}]-VIP enhanced the e.j.p. amplitude to 1.35 ± 0.15 times control ($n = 7$, $P < 0.01$) without affecting the resting membrane potential or the input membrane resistance. At higher concentrations (10^{-8} M and 10^{-7} M respectively), both agents reduced input membrane resistance in both tissues (Figure 2a, b and c, d). Figure 2c and d summarizes the effects of both VIP antagonists on the relative amplitude of the e.j.ps and input membrane resistance and on the resting membrane potential of smooth muscle cells in the cat trachea.

Effects of VIP antagonists on acetylcholine-induced contractions

As low concentrations of VIP antagonists increased the amplitude of both the e.j.ps and contractions evoked by field stimulation with no change in the membrane potential or input membrane resistance in the cat trachea, the effects of VIP-antagonists were observed on the sensitivity of smooth muscle cells of the cat tracheal tissue to acetylcholine (ACh). For this purpose, the tension development induced by various concentrations of ACh (10^{-9}–10^{-5} M) was examined in the presence of one or the other VIP antagonist. The relationship between tension development and concentration of ACh produced a sigmoidal curve for both the dog and the cat trachea, neither of which was affected by [Ac-Tyr^1, D-Phe^2]-GRF(1-29)- NH_2 or by [4-Cl-D-Phe^6, Leu^{17}]-VIP at 10^{-8} M. Figure 3a and b shows the effects of the VIP antagonists on the relationship between tension development and concentration of ACh in the cat trachea. Similar experiments were performed with 10^{-8} M [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 in the dog trachea, and again the relationship between tension development and the concentration of ACh was not affected (data not shown).

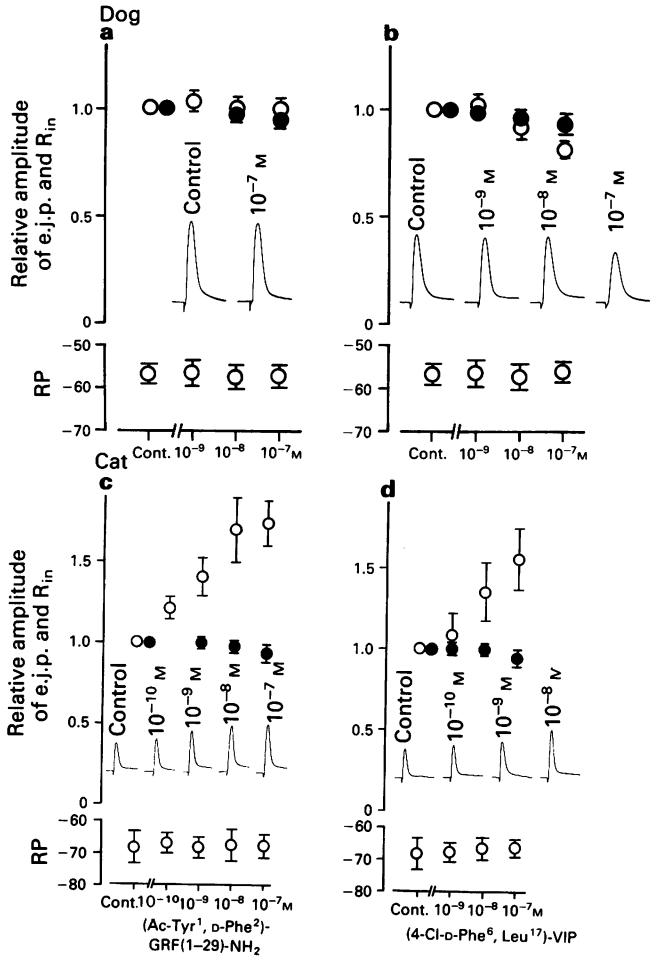

Figure 2 Relationships between the concentration of vasoactive intestinal polypeptide (VIP) antagonist and relative amplitude of e.j.p. (○), relative value of input membrane resistance (R_{in}, ●) and resting membrane potential (RP, ○) of the smooth muscle cells of a dog (a and b) and cat trachea (c and d). The amplitude of e.j.p. evoked by single field stimulation in normal Krebs solution, and the amplitude of electrotonic potentials produced by rectangular pulses in normal Krebs solution were given the relative value 1.0. Each point is the mean value derived from 5–30 experiments, and vertical bars indicate 2 × s.d. Microelectrode recording of resting membrane potential was used, and e.j.p. and electrotonic potentials were recorded by the double sucrose-gap method.

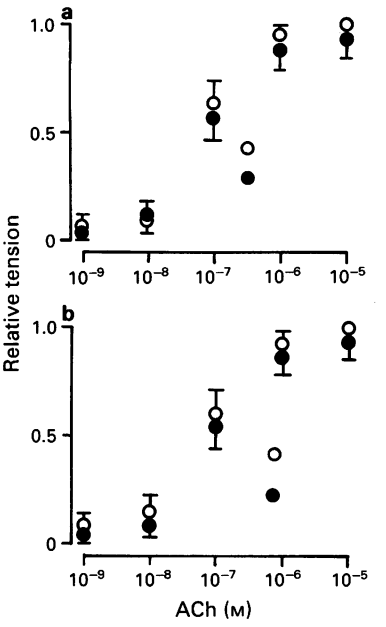

Figure 3 Effects of [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 (a) and [4-Cl- D-Phe^6, Leu^{17}]-VIP (b) on dose-response curves (acetylcholine (ACh) against tension development) of the cat trachea. Ordinate scale: relative tension development where the amplitude of contracture evoked by ACh 10^{-5} M was given the relative value 1.0. Abscissa scale: dose of ACh. Points indicate mean values with bars showing 2 × s.d. of 4–6 preparations. Data are control (○) and after treatment of the tissue with VIP-antagonists (10^{-8} M for both) (●). Absolute values of tension development induced by various concentration of ACh in the presence or absence of VIP antagonists were between 0.1 and 1.5 g.

Effects of VIP antagonists on the action of exogenous VIP on the e.j.p. amplitude

Low concentrations of VIP antagonists increased the e.j.p. amplitude with no change in the membrane potential and post-junctional response of smooth muscle cells to exogenous ACh. VIP, exogenously applied in low concentrations, has a prejunctional action, inhibiting excitatory neuroeffector transmission in the cat trachea (Hakoda & Ito, 1990). Therefore we observed the effects of VIP antagonists on the action of exogenous VIP on the e.j.p. amplitude. In the cat trachea, VIP (10^{-11} M) suppressed the e.j.p. amplitude to 0.74 ± 0.09 times the control when a single stimulus was applied with no change in the membrane potential and the input membrane resistance (data not shown). However, after the pretreatment of the tissue with the VIP antagonists [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 (10^{-8} M) and [4-Cl-D-Phe^6, Leu^{17}]-VIP (10^{-8} M), VIP (10^{-11} M) did not change the e.j.p. amplitude (to 1.02 ± 0.04 (n = 4) or to 1.01 ± 0.03 (n = 4) times control, respectively).

Effects of VIP antagonists on the e.j.ps evoked by repetitive stimulation

In parallel with the summation observed in the contractions of the dog and cat trachea, e.j.ps also showed summation when repetitive stimulation at high frequency (>10 Hz) was applied to either tissue. The summation of e.j.ps was more prominent in the dog than in the cat. Figure 4a and b shows an example of e.j.ps and contractions evoked by repetitive stimuli at 20 Hz and recorded from dog and cat tracheal tissues. In the dog trachea, the summation of e.j.ps was striking, the relative amplitude of e.j.ps evoked by 10 stimuli at 20 Hz being 21.5 ± 5.0 times the control evoked by a single stimulus (n = 4), and the relationship between the relative amplitude of e.j.ps and the number of stimulations was linear. Accordingly, the amplitude of contraction recorded simultaneously also showed marked summation (Figure 4a). In the cat trachea, on the other hand, repetitive stimuli at 20 Hz enhanced the amplitude of e.j.ps only slightly so that the e.j.p. amplitude evoked by 10 stimuli at 20 Hz was only 2.1 ± 3.0 times (n = 4) that evoked by a single stimulus, and a linear relationship between the number of stimuli at 20 Hz and the amplitude of e.j.ps was not observed.

The effects of VIP antagonists were therefore investigated on the relationship between the number of stimuli at 20 Hz and e.j.p. amplitude in dog and cat trachea. It has been reported that overnight incubation of cat tracheal tissue with [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 markedly enhances the amplitude of e.j.ps evoked by repetitive field stimulation (Hakoda & Ito, 1990). Therefore, dog tracheal tissues were treated with one or the other antagonist overnight and tracheal tissue treated with Krebs solution overnight was used for the control experiments. In the dog trachea, a linear relationship between the number of stimuli and the amplitude of the e.j.p. could be seen when several stimuli at 20 Hz were applied under control conditions (Ito & Tajima, 1981; Ito & Yoshitomi, 1988; Hakoda & Ito, 1990). Overnight treatment with either of the VIP antagonists (10^{-7} M), produced no effect on the relationship between the e.j.ps and number of stimuli at 20 Hz (Figure 4g and h). Thus, the slopes of the linear relationship between the number of stimuli and the amplitude of the e.j.ps were 2.1 ± 0.5 mV/stimulation (n = 4) and 2.2 ± 0.4 mV/stimulation respectively, after treatment with [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 (10^{-7} M) or with [4-Cl-D- Phe^6, Leu^{17}]-VIP (10^{-7} M),

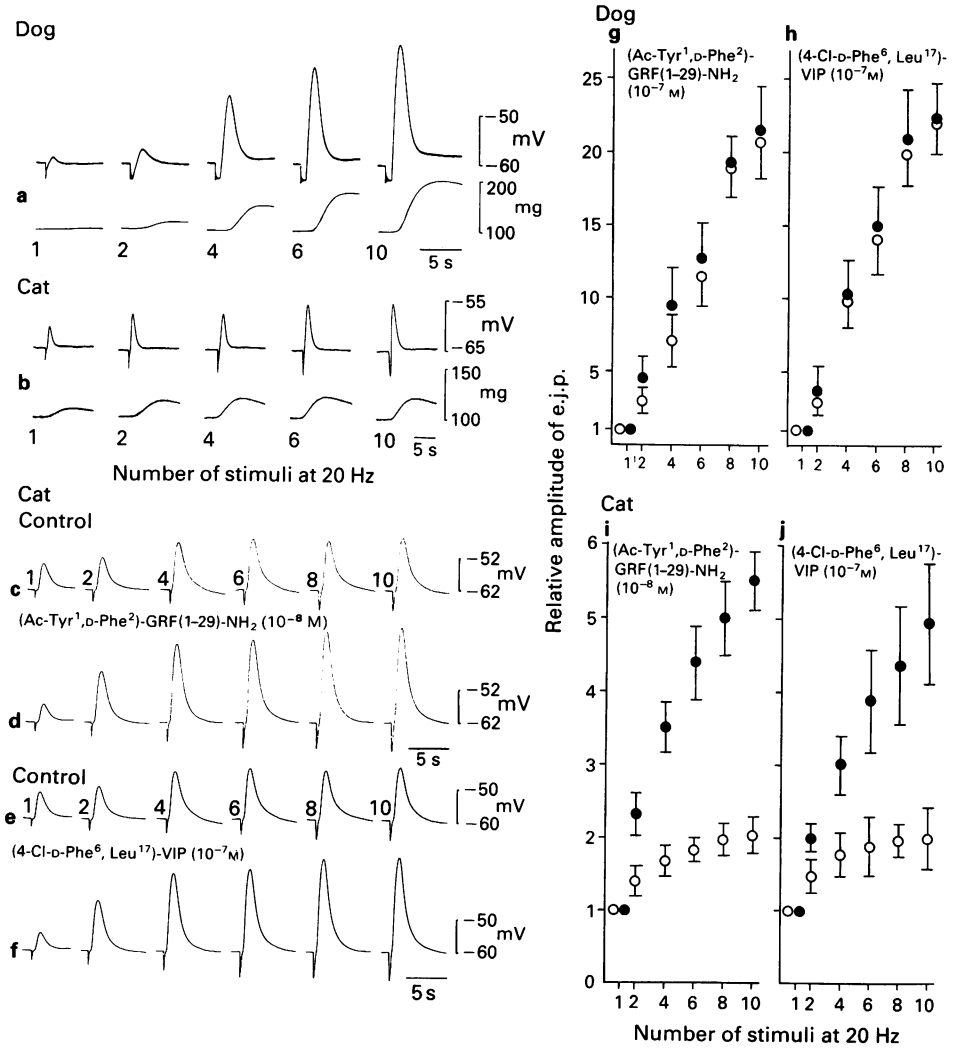

Figure 4 Effects of repetitive stimulation at high frequency (20 Hz) on the amplitude of e.j.ps and contractions of dog (a) and cat (b) tracheal tissues. (c–f) Effects of repetitive stimulation at high frequency (20 Hz) on the amplitude of e.j.ps of cat trachea in the absence (c and e) and presence of [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 (d) or [4-Cl-D-Phe^6, Leu^{17}]-VIP (f). The double sucrose-gap method was used to record e.j.ps. (g–j) Relationships between the amplitude of the e.j.p. and the number of stimuli at 20 Hz recorded from dog (g and h) and cat (i and j) in the presence (●) or absence (○) of the VIP antagonists. Each point is the mean value of 5–8 experiments, and the vertical bars indicate 2 × s.d. Absolute values of e.j.p. amplitude were between 2 and 40 mV.

the control value being 2.2 ± 0.3 (n = 5). In the cat trachea, however, treatment of the tissues with the VIP antagonists for more than 2–3 h markedly enhanced the amplitude of e.j.p. evoked by repetitive field stimulation (Figure 4c–f). After treatment of the tissue with [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 (10^{-8} M) or [4-Cl-D- Phe^6, Leu^{17}]-VIP (10^{-7} M), a linear relationship was observed, the slopes being respectively 0.6 ± 0.1 mV/stimulation (n = 8) and 0.55 ± 0.1 mV/stimulation (n = 5), indicating that the summation was enhanced by the VIP antagonists in the cat trachea (Figure 4i and j).

Discussion

The present experiments show that the VIP antagonists, [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 and [4-Cl-D-Phe^6, Leu^{17}]-VIP, act prejunctionally to enhance excitatory neuro-effector transmission presumably by enhancing ACh release from the vagus nerve in the cat trachea, since a low concentration of either antagonist significantly enhanced the amplitude of the e.j.p. and the contractions evoked by nerve stimulation without affecting the resting membrane potential, input membrane resistance or ACh-sensitivity of the smooth muscle cells. These results confirmed previous observations (Hakoda & Ito, 1990). However, in the dog trachea neither antagonist produced any effect on the amplitude of the e.j.p. or the contractions evoked by field stimulation at concentrations ranging from 10^{-9} to 10^{-8} M. It has been reported that low concentrations of exogenous VIP (10^{-11}–10^{-9} M) act prejunctionally to inhibit ACh release from the vagus nerves in the dog, cat and ferret trachea (Sekizawa *et al.*, 1988). Furthermore in the present experiments, VIP antagonists blocked the prejunctional action of exogenous VIP to suppress the e.j.p. amplitude. Therefore, it seems reasonable to assume that VIP antagonists act on the VIP receptor located at prejunctional sites, and block the inhibitory action of endogenous VIP on transmitter release in the cat trachea. The inhibitory action of endogenously released VIP on ACh release from the vagus nerve terminal would explain the less prominent summation of e.j.ps and contractions in the cat trachea than in the dog trachea. In the dog trachea, the prominent summation of e.j.ps and contraction in the presence of indomethacin, the lack of effect of VIP antagonists or of VIP antiserum (Hakoda & Ito, 1990) on excitatory neuroeffector transmission even though the cholinergic nerve fibres are also immunoreactive to VIP (Dey *et al.*, 1981) suggests that endogenous VIP may not be released in response to nerve stimulation. This view was supported by the observations that, while dog tracheo-bronchial tissues do not show NANC-induced relaxation, exogenously applied VIP

relaxed airway smooth muscle when the muscle tone was elevated with 5-hydroxytryptamine (the same authors unpublished observations). It might be that the electrical field stimulation used in the experiments was not sufficient to release the peptide from the vagal nerve terminals. It is known that classical neurotransmitters such as ACh or noradrenaline (NA) are released at low stimulus frequencies (< 10 Hz), and peptides at higher stimulus frequencies (> 20 Hz) (Bartfai *et al.*, 1988). However, VIP antagonists enhanced the e.j.p. amplitude when a single field stimulation was used to evoke e.j.p. and a single field stimulation induced NANC relaxation in the cat trachea (the same authors unpublished observations). These observations may indicate that peptides are also released at low stimulus frequencies in the cat trachea. The reason for the lack of NANC relaxation in the dog airway needs to be clarified by further experiments.

The VIP antagonists, [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 and [4-Cl-D-Phe^6, Leu^{17}]-VIP, which had been found to have a competitive action against VIP in intestinal and pancreatic cells (Waelbroeck *et al.*, 1985; Pandol *et al.*, 1986), showed no effect on the NANC-induced relaxation in the guinea-pig and cat trachea (Ellis & Farmer, 1989; Hakoda & Ito, 1990). Furthermore, Ellis & Farmer (1989) reported that these agents were without effect on the responsiveness of the guinea-pig trachea to exogenous VIP or PHI. Therefore, the VIP receptors located on the cholinergic nerve terminals may differ from those on airway smooth muscles, on the assumption that a part of NANC relaxation of the airway smooth muscles, at least, is mediated by VIP (Barnes, 1986).

At present, it is generally accepted that adrenergic neuroeffector transmission has two fundamental features, i.e., the coexistence and co-release of several transmitter substances along with NA and the negative feedback control on the NA release generally referred to as α_2-adrenoceptor autoinhibition (see, for example, Stjärne, 1989). However, it seems that these are also essential features of cholinergic neuroeffector transmission in the autonomic nervous system, since in the vagus nerve terminal, for example, peptides, such as VIP, PHI or PHM depending on species, are co-released and ACh release is modulated in many ways. For example, prejunctional inhibition of the release of ACh from the vagal nerve terminals has been noted with ACh, NA, prostaglandins and VIP, whereas mediators such as histamine, 5-hydroxytryptamine and substance P enhance the release of ACh by prejunctional mechanisms (Ito, 1990). Airway smooth muscle tissues receive a rich cholinergic innervation (Richardson, 1979), and ACh is one of the most potent agents causing bronchoconstriction. This being the case, investigations on modulatory mechanisms involved in the release of ACh from the vagus nerve terminal would seem to be particularly important.

In the cat trachea, VIP and ACh co-exist in the same nerves (Lundberg, 1981; Dey *et al.*, 1981), and VIP may be released as a co-transmitter (Barnes, 1986). VIP, when released, directly relaxes the airway smooth muscle without changing the electrical membrane properties of the smooth muscle cells (Ito & Takeda, 1982), and probably inhibits the release of ACh from the nerve, thus playing a 'double-braking' role on bronchoconstriction. On the other hand, in the dog airway smooth muscle, it has been reported that e.j.ps and twitch contractions elicited by nerve stimulation show a gradual and continuous reduction in amplitude during perfusion with normal physiological solution (Ito & Tajima, 1981; Walters *et al.*, 1984; Inoue & Ito, 1986). The prostaglandin synthesis inhibitor, indomethacin, and the prostaglandin antagonist, SC-19220, suppress the decline of the e.j.ps and twitch contractions evoked by nerve stimulation. During the action of indomethacin or SC-19220, no change in the membrane potential, membrane input resistance or sensitivity to ACh of the smooth muscle was observed, indicating that these chemicals are acting on the cholinergic nerve terminals in the muscle tissue. When the amplitude of e.j.ps or twitch contractions evoked by field stimulation were stabilized in the presence of indomethacin, prostaglandin E series in very low concentrations (10^{-12}–10^{-11} M) reduced the amplitude of twitch contractions and e.j.ps. Furthermore, the amount of prostaglandin E released into the perfusate was increased by field stimulation to 3–4 times that observed in the absence of stimulation. This increase would explain the decremental responses of the e.j.ps and twitch contractions (Inoue *et al.*, 1984; Ito, 1991). Furthermore, subcutaneous injection of indomethacin has been shown to induce wheezing or coughing in the dog (Ito & Tajima, 1981). These observations strongly indicate that inhibitory mechanisms mediated by endogenous prostaglandins on ACh release from the nerve terminals of the vagus play important roles in the regulation of the motility of tracheal and bronchial muscle in the dog. However, it appears that the mechanisms involved in the modulation of ACh release from the vagus nerve terminals are different in different species.

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