Br. J. Pharmac. (1983), **79**, 634–636

Evidence against vasoactive intestinal polypeptide (VIP) as a dilator and in favour of substance P as a constrictor in airway neurogenic responses

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Propranolol-resistant neurogenic relaxation persisted in (carbachol-contracted) guinea-pig tracheae already relaxed by supramaximal concentrations of vasoactive intestinal polypeptide (VIP). Also, VIP relaxed preparations that were under neurogenic inhibition. In hilus bronchi, about 60% of a neurogenic contraction was atropine-resistant. (Arg^5, D-Trp^{7,9}) SP_{5-11} specifically antagonized this contraction and those produced by exogenous substance P. Substance P, but not VIP, seems to be involved in nerve-mediated effects on guinea-pig airway tone.

Introduction Putative neurotransmitters for neural, atropine-resistant contraction and propranolol-resistant relaxation of guinea-pig airways have been proposed on the basis of immunohistochemical studies. Thus, substance P and vasoactive intestinal polypeptide (VIP) are present in nerve fibres in airways (Nilsson, Dahlberg, Brodin, Sundler & Strandberg, 1977; Uddman, Alumets, Densert, Håkanson & Sundler, 1978). Matsuzaki, Hamasaki & Said (1980) showed that the relaxant peptide, VIP, was released on nerve stimulation in guinea-pig trachea and that incubation of trachea with antibodies to VIP antagonized a neurogenic relaxation. However, the specificity for inactivation of endogenous VIP by the antibodies was not established. Furthermore, Matsuzaki *et al.* (1980) obtained surprisingly small mean relaxations (35 mg in tracheae with a tone of about 1000 mg) and diminutive responses (5 mg) were included in their calculations. We have examined responses to VIP, the effects of inhibitory nerve stimulation and the interaction between these two interventions in contracted guinea-pig tracheal preparations.

Grundström, Andersson & Wikberg (1981) found that nerve-induced contraction of guinea-pig hilus bronchi was entirely atropine-resistant. With antagonists to the contractile peptide, substance P, now available (Folkers, Hörig, Rosell & Björkroth, 1981), it was of interest to study the interaction between an antagonist and this neurogenic contraction. In isolated, electrically contracted hilus bronchi, the effect of a heptapeptide substance P antagonist (Bynke, Håkanson, Hörig & Leander, 1983) has been studied.

Methods Tracheal rings from guinea-pigs (body weight 300–500 g) (see Karlsson & Persson, 1981) were mounted in 2.5 ml organ baths containing Krebs solution (gassed with 95% O_2 and 5% CO_2) for recording of isometric tension changes (Grass FTO3, Grass polygraph model 5). Tubal segments were taken from the hilus bronchi of the left and right caudal lobes and mounted as described above (initial tension 0.3 g). Tracheal and bronchial preparations were electrically stimulated (Grass S88 stimulator) using field stimulation via platinum electrodes. A supramaximal voltage was used which produced responses that were blocked by tetrodotoxin (1 μg/ml). When studying inhibitory nerve-stimulation in tracheae, propranolol (0.1 μM) was always present. Mean ± s.e.mean values from preparations obtained from different animals are given.

Drugs used were: atropine sulphate (Sigma), carbachol chloride (Sigma), propranolol dihydrochloride (Hässle), (Arg^5, D-Trp^{7,9}) substance P_{5-11} (gift from Dr Hörig, Ferring), tetrodotoxin (Sankyo) and vasoactive intestinal polypeptide (VIP, V. Mutt.). All drugs were dissolved in 0.85% NaCl solution.

Results

Relaxation and vasoactive intestinal polypeptide

Tracheal preparations developed a spontaneous tone of 1780 ± 233 mg ($n = 9$). Maximum relaxation induced by a train of stimuli for 2 min (pulse duration 0.5 ms and frequency 20 Hz) reduced tracheal tone by 1314 ± 130 mg (in the presence of 0.1 μM propranolol). This relaxation is considerably larger than that reported by Matsuzaki *et al.* (1980).

Maximum effective concentrations of VIP (23.0 μM) reduced the tone in carbachol-contracted tracheal preparations by 29.8 ± 4.1% ($n = 4$). A comparable maximal relaxation, 24.5 ± 2.9% ($n = 8$), was produced by nerve stimulation, using the same stimulus parameters as above. Carbachol 5.5×10^{-6} M was used. This concentration raised the tone to 2620 ± 200 mg ($n = 12$) and corresponded to carbachol's EC_{95}. In carbachol-contracted tracheal preparations with a supramaximal concentration of

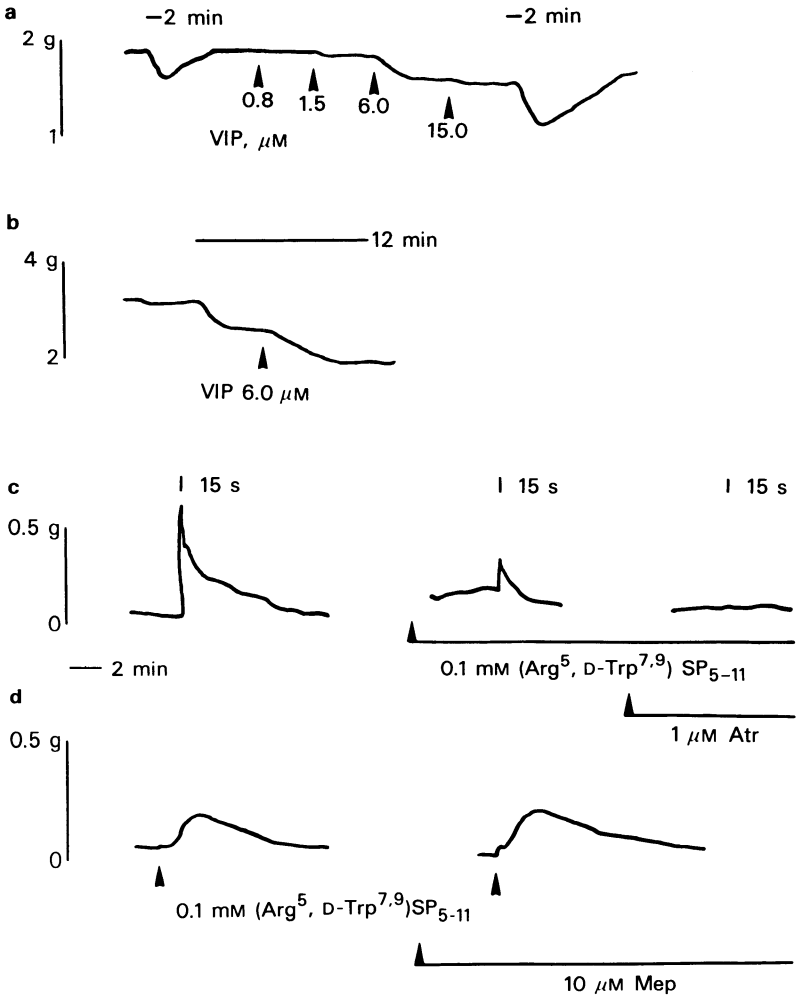

Figure 1 (a) In a carbachol-contracted (5.5 μM) tracheal ring in the presence of propranolol (0.1 μM), electrical field stimulation (horizontal lines above the tracing) produces a relaxant effect that is not changed by the presence of maximally effective concentrations of vasoactive intestinal polypeptide (VIP). **(b)** In a carbachol-contracted (5.5 μM) tracheal ring preparation already relaxed by prolonged electrical stimulation (horizontal line), VIP is still an efficient relaxant (0.1 μM propranolol present). **(c)** In a tubal segment of hilus bronchi electrical stimulation (vertical lines) produces contraction, the major part of which is antagonized by a heptapeptide substance P antagonist. The minor part is antagonized by atropine (Atr). Time calibration (2 min) is shown to the left of the figure. **(d)** A transient contraction is produced by the substance P antagonist in a bronchial preparation. The contraction is not attenuated by mepyramine (Mep). Time calibration as in (c).

VIP present (VIP was added until no further relaxation was recorded and then its concentration was doubled) the inhibitory response to nerve stimulation was intact (Figure 1a). Moreover, the mean relaxation induced by 6.0 μM VIP (19.9 ± 6.5%, $n = 5$; corresponding to the EC_{70}) was unchanged (18.7 ± 3.2%, $n = 5$) when evaluated during a maximal inhibitory response to prolonged nerve stimulation (Figure 1b).

Contraction and substance P Electrical stimulation using a train of stimuli for 15 s (pulse duration 0.6 ms and frequency 15 Hz) of the hilus bronchi produced reproducible contractile responses. Atropine, 1 μM, reduced these by 37.7 ± 4.9% ($n = 9$). Atropine-resistant contractions had a mean tension of 201 ± 36 mg ($n = 13$).

Treatment of bronchial preparations with (Arg^5, D-$Trp^{7,9}$) SP_{5-11} for 15 min reduced the magnitude of

the electrically induced contraction, the residual response being completely blocked by atropine (Figure 1c). In the presence of atropine (1 μM) (Arg^5, D-Trp^{7,9}) SP_{5-11} 10^{-5} M, 3×10^{-5} M and 10^{-4} M inhibited the amplitude by $16.2 \pm 6.9\%$ ($n = 5$), $63.5 \pm 8.2\%$ ($n = 5$) and $77.1 \pm 3.4\%$ ($n = 8$), respectively.

(Arg^5, D-Trp^{7,9}) SP_{5-11} (10^{-5} M to 10^{-4} M) produced a transient (< 15 min) contractile response in 16 of 36 preparations. Treatment with mepyramine (3–10 μM) did not affect this contraction (Figure 1d).

The concentration-response relationship to histamine, carbachol, and substance P was examined in the absence and presence of 10^{-4} M (Arg^5, D-Trp^{7,9}) SP_{5-11}. Only substance P-induced contractions were antagonized. Its concentration-response line was shifted to the right 2.3 times ($P < 0.05$, two-tailed Student's t test). The concentration-response lines to histamine and carbachol tended to be shifted to the left although this was not statistically significant ($P > 0.05$). The following EC_{50} values were obtained; histamine ($n = 3$) 14.6 ± 4.8 μM and 8.2 ± 0.8 μM, carbachol ($n = 4$) 1.65 ± 0.61 μM and 0.83 ± 0.19 μM, substance P ($n = 3$) 26.3 ± 6.4 μM and 62.7 ± 20.2 μM in the absence and presence, respectively, of the antagonist.

Discussion In the presence of maximally effective concentrations of VIP the neurogenic inhibition of carbachol-contracted tracheae persisted, suggesting that the relaxation induced by nerve stimulation is unrelated to VIP. Against this interpretation it could perhaps be argued that a neurotransmitter, when added exogenously, may not reach subsynaptic receptors because of diffusion barriers and thus be less effective than stimulation of nerves. This argument may not be valid, however, because the effect of VIP was unchanged when the peptide was added during prolonged electrical stimulation, i.e. when subsynaptic receptors might be expected to be fully activated.

In preparations of hilus bronchi about 60% of the magnitude of the neurogenic contractions was atropine-resistant. This finding suggests that both non-cholinergic (Grundström *et al.*, 1981) and cholinergic neural contractions can be induced in these bronchi. The non-cholinergic contraction was antagonized by (Arg^5, D-Trp^{7,9}) SP_{5-11} in a concentration-dependent way. This peptide transiently contracted some preparations via a mechanism apparently unrelated to histamine release since it was mepyramine-resistant. Some specific antagonism by (Arg^5, D-Trp^{7,9}) SP_{5-11} of contractions induced by exogenous substance P was also shown. The data thus support a mediator role for substance P as well as acetylcholine in neurogenic contraction of the hilus bronchi.

During the preparation of this communication we have become aware of studies from two other laboratories. Håkanson and Leander (personal communication) observed that a substance P antagonist inhibited atropine-resistant neurogenic contractions in guinea-pig stem bronchi, while Lundberg, Saria, Brodin, Rosell & Folkers (1983) showed that in the presence of mepyramine, an undecapeptide substance P antagonist completely antagonized neurogenic contractions of guinea-pig hilus bronchi.

Thus, this study of guinea-pig airways provides evidence against a role for VIP as a neurogenic dilator of the trachea, but supports a role for substance P and acetylcholine as neurally released constrictors of hilus bronchi.

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(*Received February 25, 1983.*)