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Suppression by neuropeptide Y of capsaicin-sensitive sensory nerve-mediated contraction in guinea-pig airways

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1 In the present study we have examined whether neuropeptide Y (NPY) interferes with non-adrenergic, non-cholinergic nerve-mediated contractions and relaxations in the guinea-pig airways. In these experiments we have used ring preparations of bronchi and trachea, incubated in the presence of atropine, propranolol and indomethacin (each 1 μM).

2 The contractile response to electrical stimulation of non-adrenergic, non-cholinergic nerve fibres was suppressed by NPY and NPY 13–36 in a concentration-dependent manner, these agents having similar inhibitory potencies. NPY caused a more complete inhibition than the C-terminal fragment.

3 NPY affected neither the basal tension nor the substance P-evoked contraction in the bronchi and trachea and did not interfere with nerve-mediated, non-adrenergic relaxation in the trachea.

4 On the basis of these results, it is suggested that NPY may act on the terminals of sensory neurones in the airways to prevent antidromic, excitatory neurotransmission by inhibiting transmitter release.

Introduction

Neuropeptide Y (NPY) is a peptide containing 36 amino acids. It has a wide distribution in the peripheral nervous system. It is present in many sympathetic neurones where it is co-localized with noradrenaline (NA). NPY causes vasoconstriction *per se* and enhances NA-evoked vasoconstriction (for a review see Edvinsson *et al.*, 1987). The effects of NPY are mediated via postjunctional receptors, tentatively referred to as Y_1-receptors (Wahlestedt *et al.*, 1986). In addition, NPY suppresses the stimulated release of NA from sympathetic nerve terminals. This effect is prejunctional and mediated via Y_2-receptors which can be activated by the C-terminal 13–36 fragment of NPY, which is not recognized by the Y_1-type receptor (Wahlestedt *et al.*, 1986; 1987).

In the tracheobronchial wall, NPY fibres are fairly numerous around arteries, seromucous glands and in the smooth muscle (Uddman *et al.*, 1984). NPY has been shown to suppress parasympathetic nerve activity in the trachea (Grundemar *et al.*, 1988; Stretton & Barnes, 1988), uterus (Stjernquist *et al.*, 1983), heart (Potter, 1985) and ileum (Garzon *et al.*, 1986). Electrical field stimulation of guinea-pig bronchi elicits nerve-mediated contractions, that consist of an atropine-sensitive and an atropine-resistant component (Grundström *et al.*, 1981). The latter response is mediated by excitatory non-adrenergic, non-cholinergic (excitatory NANC) nerves. NA is known to suppress these contractions by an action on prejunctional α_2-adrenoceptors (Grundström *et al.*, 1984; Grundström & Andersson, 1985). Electrical field stimulation of the guinea-pig trachea elicits nerve-mediated relaxations that also can be divided into two components. Part of the response is sensitive to antagonists at β-adrenoceptors and to adrenergic neurone-blocking agents. The remaining component is mediated by inhibitory non-adrenergic, non-cholinergic (inhibitory NANC) nerves (Coburn & Tomita, 1973; Grundström *et al.*, 1981).

The purpose of the present sutdy was to see whether NPY interferes with excitatory or inhibitory NANC neurotransmission in the guinea-pig airways.

Methods

Male guinea-pigs weighing 300–600 g were used. The lungs including the trachea and the bronchi were removed and placed in Krebs solution equilibrated with 95% O_2 and 5% CO_2. Ring segments, 2–3 mm in length, were obtained from the upper part of the main bronchi and from the lower part of the trachea. The preparations were mounted on special holders (Grundström *et al.*, 1981) for measurement of isometric tension and placed in a 5 ml organ bath containing Krebs solution at 37°C. After an equilibration period of 60–90 min a resting tension of 1.5–3 mN was attained. Platinum wire electrodes were placed on either side of the preparation and a Grass S88 stimulator was used to produce electrical field stimulation. Monophasic square waves of 1 ms duration at 40 V and 2 Hz were used. The pulse trains had a duration of 30 s and were delivered once every 15 min when contractions were studied and once very 5 min when relaxations were studied. The buffer solution contained propranolol 1 μM and atropine 1 μM. Indomethacin 1 μM was added because it improves the reproducibility of the contractile response to electrical stimulation (Szolcsányi & Barthó, 1982). The peptides were added cumulatively with each concentration increment being given 10–12 min before the next stimulated contraction.

The pD_2 or $-\log(EC_{50})$ values were estimated from the concentration-response curves by fitting the logistic function (Parker & Waud, 1971) to the experimental data by means of non linear regression analysis (Marquardt, 1963). Data are given as mean values ± s.e.mean except when pD_2 values are presented; these are instead accompanied by 95% confidence limits. Statistical significance were tested by means of analysis of variance.

The following drugs were used: indomethacin, capsaicin and tetrodotoxin (TTX) were purchased from Sigma Chemical Co., St Louis, MO, U.S.A.; porcine NPY and substance P (SP) (Peninsula, Belmont, CA, U.S.A.); porcine NPY 13-36 (Ferring AB, Malmö, Sweden); atropine sulphate (ACO, Solna, Sweden); propranolol hydrochloride (Du Pont Meda, U.S.A.); idazoxan (Reckitt & Colman, Hull, England).

The Krebs solution had the following composition (mM): NaCl 122, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 15.4, KH_2PO_4 1.2 and glucose 5.5. Indomethacin was dissolved in 5% $NaHCO_3$ in distilled water to a concentration of 1 mM. All other drugs were dissolved in and diluted with distilled water.

Results

Electrical field stimulation of guinea-pig bronchial rings evoked a rapid twitch, followed by a more long-lasting contraction with a duration of 10–15 min. Addition of atropine

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(1 μM) abolished the rapid response. Addition of TTX (1 μM) or capsaicin (1 μM) virtually abolished the atropine-resistant contraction (Figure 1).

In subsequent studies, the preparations were allowed to equilibrate for 90 min before a series of atropine-resistant contractions were elicited; the second contraction (which amounted to 5.0 ± 0.9 mN, $n = 10$) in a series was set as 100%. With indomethacin in the bath the non-cholinergic contractions were reproducible for at least 3 h.

Neither NPY nor NPY 13–36 had any effect on the basal tension. Electrically induced atropine-resistant contractions were inhibited by NPY and NPY 13–36 in a concentration-dependent manner (Figures 2 and 3).

Maximally effective concentrations of NPY and NPY 13–36 reduced the responses to 17.5 ± 3.4% and to 68.5 ± 3.4% of control contractions, respectively. Both peptides showed a similar inhibitory potency; the estimated pD_2 values are shown in Table 1. Pretreatment with 1 μM idazoxan, a selective α_2-adrenoceptor antagonist (Doxey *et al.*, 1983), did not affect the NPY-evoked suppression of the electrically evoked contractile response ($n = 5$). As expected, idazoxan (1 μM) suppressed the NA-evoked inhibition of the stimulated atropine-resistant contractions (not shown in figure). SP, the proposed neuromessenger in excitatory NANC fibres induced a contraction (at a concentration of 1 μM) which roughly matched the electrically evoked, atropine-resistant response. The SP-evoked contractions were reproducible and the second response following the equilibration period was used as control. NPY (0.3 μM) did not affect these responses (Figure 4).

The possibility that NPY might suppress the inhibitory NANC neurotransmission of guinea-pig airways was also

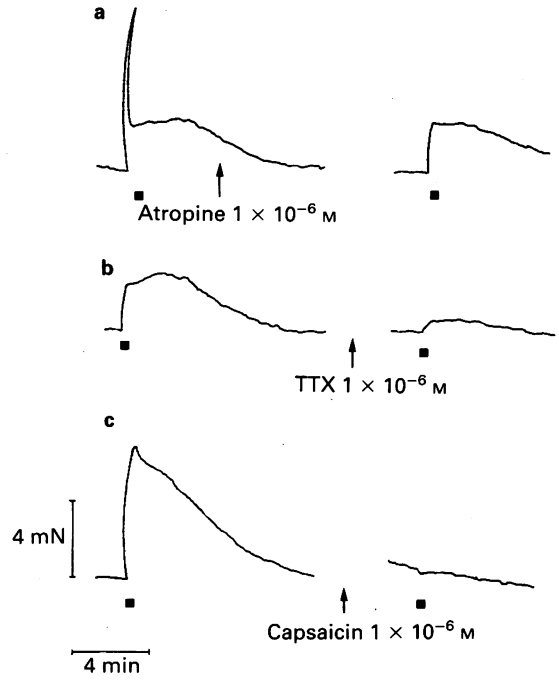

Figure 1 Guinea-pig isolated bronchial ring: electrical field stimulation (EFS), (1 ms, 40 V and 2 Hz) indicated below the recordings, evokes a rapid twitch and a slow contraction. (a) Exposure to atropine (10 min) blocks the twitch but is without effect on the slow contraction. (b) EFS on an atropinized preparation evokes a slow contraction that is abolished by adding tetrodotoxin (TTX) to the bath (30 min). (c) The contractile effect of EFS on an atropinized preparation is abolished by adding capsaicin to the bath (60 min).

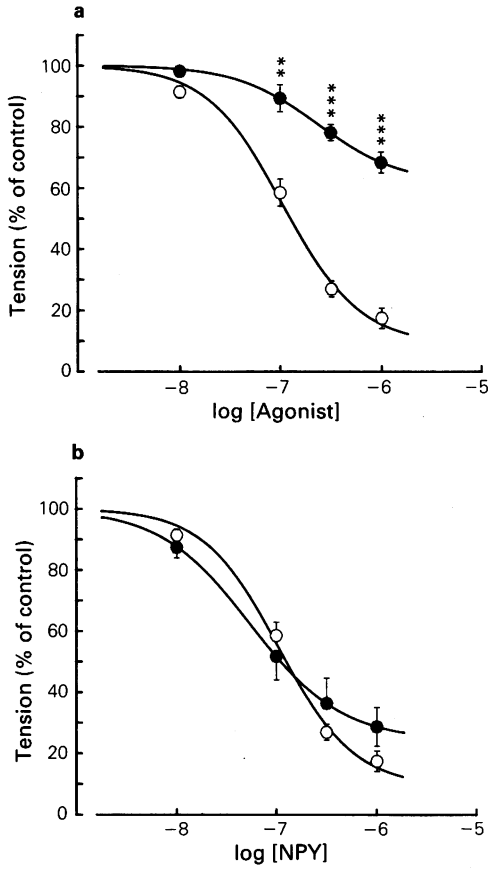

Figure 3 Guinea-pig isolated bronchial ring: log concentration-response curves for the inhibitory effects of neuropeptide Y (NPY) and NPY 13–36 on the electrically induced non-cholinergic contractions. In (a) the effects of NPY (○) and NPY 13–36 (●) are shown. In (b) the effect of pretreatment with 1 μM idazoxan (●) on NPY-induced inhibition is shown; the NPY control curve (○) is the same as in (a). The significance of the differences between the observations of the NPY control curve and the other two curves was tested by analysis of variance. Significances are denoted by ** = $P < 0.01$ and *** = $P < 0.001$. Vertical bars denote s.e.mean ($n = 4$–6).

Table 1 Estimated pD_2-values for the inhibitory effect of neuropeptide Y (NPY) and NPY 13–36 on electrically induced non-cholinergic contraction of guinea-pig bronchial rings

Substances	pD_2	*(95% confidence limits)*	*n*
NPY	7.0	(6.8–7.2)	6
NPY 13–36	6.6	(6.0–7.3)	4
NPY + idazoxan	7.3	(6.8–7.7)	6

n = number of experiments.

Figure 2 Guinea-pig isolated bronchial ring: a representative recording illustrating the effect of neuropeptide Y (NPY) on the nerve-mediated contractions of bronchi in the presence of atropine (1 μM) and propranolol (1 μM). Indomethacin (1 μM) was also present throughout. Electrical field stimulations (parameters as in Figure 1) are indicated above the recording.

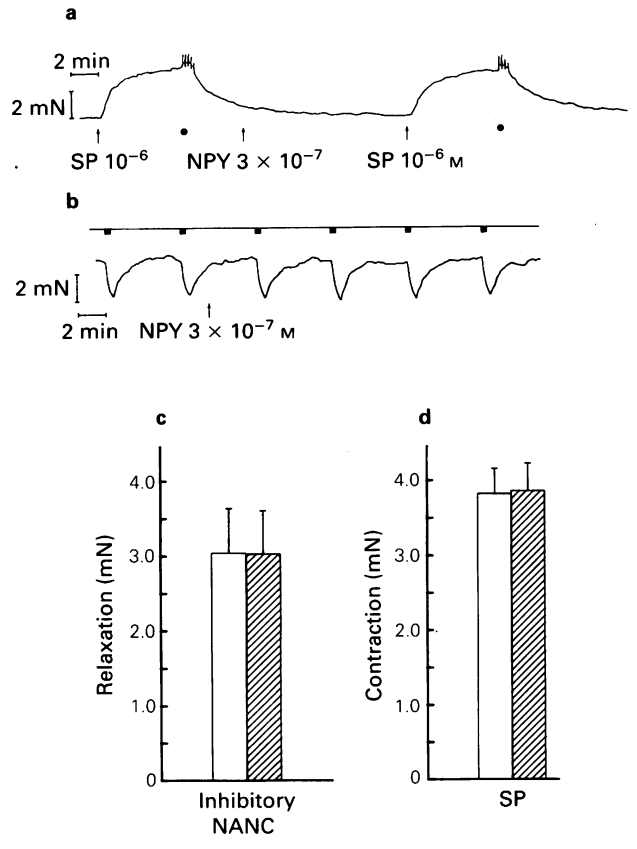

Figure 4 (a) Nerve-mediated non-adrenergic, non-cholinergic (NANC) relaxation of guinea-pig trachea before and after addition of neuropeptide Y (NPY). (b) Substance P (SP)-evoked contraction of the bronchi before and after incubation of NPY. (● indicates rinsing of the bath). NPY had no effect on either SP-induced contractions or on electrically evoked relaxations. (c and d) Data summarizing the results of all experiments performed as in (a) and (b), respectively ($n = 5$). Vertical lines give s.e.mean. Open columns = control responses; hatched columns = responses observed after treatment with NPY (3×10^{-7} M).

investigated. The tracheal preparations were equilibrated in the presence of propranolol, atropine and indomethacin and were contracted with 0.3 μM histamine which gave a stable tension of 18 ± 4.4 mN ($n = 5$). Electrical field stimulation then elicited reproducible relaxations. The relaxation preceding the addition of NPY was set as 100 and the second relaxation after the addition of NPY was used to evaluate the possible effect. The non-adrenergic relaxations were unaffected by 0.3 μM NPY (Figure 4).

Discussion

NPY has a number of effects outside the sympathetic neuroeffector junction. It has for instance been shown to inhibit nerve-mediated (cholinergic) contractions of the isolated vagus-nerve trachea preparation (Grundemar *et al.*, 1988) and of tracheal ring preparations (Stretton & Barnes, 1988). The results of the present study suggest that NPY is capable of suppressing not only parasympathetic activity in the guinea-pig airways but also neurotransmission in excitatory NANC nerve fibres. It is quite likely that these nerve fibres are identical with sensory nerves of the C-fibre type, which employ SP and/or related tachykinins as transmitters. The contractile response evoked by these fibres upon electrical stimulation is inhibited by tachykinin antagonists (Lundberg *et al.*, 1983; Leander *et al.*, 1984) and the response is abolished by pretreatment with capsaicin (Figure 1; Lundberg & Saria, 1982) (for a recent review see Holtzer, 1988). NPY 13–36 was also effective, although less so than the whole molecule. NPY had no effect on basal tension or on SP-evoked contractions and NPY had no effect on the inhibitory NANC neurotransmission in the trachea. The present data indicate therefore that NPY acts on the sensory nerve terminals, rather than on the terminals of NANC inhibitory neurones or on the smooth muscle cells of the airways.

In the central nervous system there is evidence for an interaction between NPY and α_2-adrenoceptors. It has been suggested that NPY upregulates α_2-adrenoceptors in the medulla oblongata in the rat (Agnati *et al.*, 1983). Idazoxan inhibits NPY-induced suppression of spontaneous locomotor activity in the rat (Heilig *et al.*, 1988). Like NPY, NA inhibits electrically stimulated, sensory nerve-mediated, atropine-resistant contractions in the guinea-pig bronchi (Grundström *et al.*, 1984). However, NA acts via α_2-adrenoceptors on the sensory nerve terminals. The results of the present study indicate that the action of NPY on sensory neurotransmission in the airways does not involve α_2-adrenoceptors.

NPY seems to act on both cholinergic and NANC (sensory) excitatory nerves in the airways. The inhibitory NANC-nerves of the airways are not affected. The involvement of excitatory nerves in asthmas is a subject of considerable interest (See Andersson & Grundström, 1987; Nadel & Barnes, 1987). The possibility that nerve-mediated obstruction of the airways can be prevented by interfering with NPY-receptors or α_2-adrenoceptors, may offer new approaches for treatment.

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