Antagonism of the inhibitory action of neuropeptide Y (NPY) in guinea-pig trachea by the C-terminal fragment NPY (2-36)

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The C-terminal fragment of neuropeptide Y (NPY), NPY(2-36) was used as a means of discriminating between two differently located NPY receptor sites in guinea-pig trachea. Both NPY and NPY(2-36) reduced the maximal relaxation elicited by vasoactive intestinal peptide (VIP). In contrast, the C-terminal fragment did not mimic the inhibitory action of NPY on the noradrenaline (NA) evoked response. However, pretreatment of the trachea with 30 nM NPY(2-36), 5 min before generating NA and VIP concentration-response curves in the presence of NPY, abolished the inhibitory effect of NPY on NA-elicited response but did not affect the modulatory action of NPY on VIP-induced relaxation. These results suggest that the two differently located NPY receptor sites in guinea-pig trachea are of two distinct subpopulations.

Keywords: Neuropeptide Y (NPY); airways; neuropeptide Y(2-36); antagonism

Introduction Neuropeptide Y (NPY) has been shown to exert a broad variety of pharmacological actions including central, hormonal and peripheral effects (Dumont *et al.*, 1992). Agreement exists between authors that in the lung, NPY influences airway calibre by interfering with the release of transmitters both from non-adrenergic, non-cholinergic and sympathetic nerves (Grundemar *et al.*, 1990; Stretton *et al.*, 1990). Recently, we have shown that NPY is also capable of regulating airway smooth muscle tone by modulating the effector response of vasoactive intestinal peptide (VIP) and noradrenaline (NA) via two different mechanisms of action (T.-Benchenkroun *et al.*, 1992). Whether this non-specific inhibitory action of NPY results from interaction with two different NPY receptor subtypes is unknown.

Results from structure activity studies have revealed that the full expression of the effects of NPY in most systems depends upon both intact C- and N-terminals (Dumont *et al.*, 1992). An interesting exception to this rule was the observation that NPY(2-36), injected intracerebroventricularly was more potent than NPY in increasing food intake whereas it was less potent than the native peptide in decreasing body temperature (Jolicœur *et al.*, 1991). Since prominent differences were found between these two effects of NPY(2-36), these findings were used to address the possible existence of different NPY receptor subtypes in the central nervous system (Stanley *et al.*, 1992). In the present study, NPY(2-36) was used to explore further potential differences in NPY receptors mediating the modulatory influence of NPY on responses to NA and VIP in guinea-pig trachea.

Methods Tracheae from Hartley albino guinea-pigs were set up for isometric recording (Grass Instruments) in 5 ml organ baths (Krebs solution) under a resting tension of 1 g. Cumulative concentration-response curves for VIP and NA were generated either in the absence or in the presence of 30 nM (5 min) NPY or NPY (2-36). To evaluate the antagonistic properties of NPY(2-36), 30 nM of the C-terminal fragment was added to the organ bath 5 min before generating VIP or NA concentration-response curves in the presence of NPY. All data are given as means $\pm$ s.e.mean.

The composition of the Krebs solution was as follows (mM): NaCl 118.1, KCl 4.7, MgSO₄ 7H₂O 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, CaCl₂ 2.5 and glucose 11.1. Porcine (p)-NPY(1-36), pNPY(2-36) and VIP were synthesized in our labor-

atories. Noradrenaline ((-)-arterenol hydrochloride) (NA) was purchased from Sigma Chemical Co. (St. Louis, U.S.A.). Other experimental conditions and data analysis methods were the same as those described previously by T.-Benchenkroun *et al.* (1992).

Results Mean maximal amplitudes of responses induced by VIP (10^{-10} to 10^{-6} M) and NA (10^{-8} to 6×10^{-5} M) reached 0.59 ± 0.05 and 0.59 ± 0.06 g, respectively. As demonstrated earlier (T.-Benchenkroun *et al.*, 1992), preincubation of tracheae with 30 nM NPY (5 min) resulted in significant reductions of the maximal response to VIP and NA (by 37.2 and 40.6%, respectively) without affecting their pD₂ values (Figure 1a,b; Table 1).

C-terminal fragment NPY(2-36) had no effect on its own on the resting tone of the trachea. When added 5 min before generating VIP or NA concentration-response curves, NPY(2-36) reduced by 35.5% ($P < 0.05$) the maximal response induced by VIP whereas it did not cause any change in the magnitude of the relaxation elicited by NA. Furthermore, in neither case, were the pD₂ values of both agonists altered in the presence of NPY(2-36) (Table 1). However, as shown in Figure 1a and b, following a 5 min pretreatment of the tracheae with 30 nM NPY(2-36), NPY failed to reduce the NA-evoked maximal response but maintained its inhibitory influence on VIP-induced relaxation. However, the magnitude of the inhibitory effect of NPY on the response to VIP in the presence of NPY(2-36) was similar to that obtained with NPY alone, and averaged 45.7%. Maximal responses and pD₂ values for both agonists in the presence of NPY plus NPY(2-36) are summarized in Table 1.

Discussion The present results confirm previous observations that NPY exerts a potent inhibitory action on VIP- and NA-induced relaxation in guinea-pig trachea (T.-Benchenkroun *et al.*, 1992). Moreover, our results provide further evidence of the importance of the N-terminal portion of the NPY molecule for adequate interaction with its receptors and for the maintenance of its full biological activity (Dumont *et al.*, 1992). Deletion of the Tyr¹ of NPY resulted in the formation of a peptide that behaved differently from NPY as a modulatory agent on the relaxation induced by the two agonists. NPY(2-36) mimicked to a similar degree the effect of NPY on VIP-evoked responses whereas it was totally inactive on NA-elicited relaxation. This finding that NPY(2-36) may be as active as NPY in the trachea is similar to what has been observed in behavioural studies (Jolicœur *et al.*, 1992; Stanley *et al.*, 1992) and is in line with previous reports

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Figure 1 Effects of neuropeptide Y(2-36) (NPY(2-36), $\square$) on the inhibitory action of NPY ($\bullet$) on (a) vasoactive intestinal peptide (VIP)- and (b) noradrenaline (NA)-induced cumulative concentration-response curves in guinea pig trachea; ($\circ$) VIP (a) or NA (b) alone. NPY(2-36) (30 nM) was added to the organ bath 5 min before the administration of NPY (30 nM) which was also added 5 min prior to (a) VIP or (b) NA. Responses are expressed as absolute grams of relaxation. Means are shown with s.e.mean. * $P < 0.05$ as compared with control values (VIP or NA alone) by use of Student's t test for unpaired data.

Table 1 Maximal responses (m.r.) and pD_2 values for vasoactive intestinal peptide (VIP) and noradrenaline (NA) in guinea-pig trachea in the absence or presence of neuropeptide Y (NPY, 30 nM) and/or C-terminal fragment NPY(2-36) (30 nM)

Pretreatment	m.r. (g)	pD_2
Control VIP	0.59 ± 0.05	7.55 ± 0.09
NPY + VIP	0.37 ± 0.02^a	7.54 ± 0.06
NPY(2-36) + VIP	0.38 ± 0.04^a	7.50 ± 0.08
NPY(2-36) + NPY + VIP	0.32 ± 0.06^a	7.50 ± 0.1
Control NA	0.59 ± 0.06	5.92 ± 0.05
NPY + NA	0.35 ± 0.06^a	6.04 ± 0.09
NPY(2-36) + NA	0.57 ± 0.06	6.16 ± 0.02
NPY(2-36) + NPY + NA	0.59 ± 0.09	5.85 ± 0.06

The m.r. and pD_2 values are expressed as means $\pm$ s.e.mean of data obtained from 4 to 14 tissues.

* $P < 0.05$ as compared with control responses (VIP or NA alone) by means of Student's t test for unpaired data.

in which various *in vitro* pharmacological effects of NPY and its related fragments have been measured (Rioux *et al.*, 1986; Donoso *et al.*, 1988). However, the interesting observation of the present study is that the C-terminal fragment NPY(2-36) acted as a full antagonist of NPY on NA-induced relaxation. To our knowledge, it is the first time that such antagonistic properties of NPY(2-36) have been observed.

We have previously reported that the inhibitory action of NPY in guinea-pig trachea is expressed following interaction with both pre- (NA) and post-junctional (VIP) receptors (T.-Benckroun *et al.*, 1992). These receptors were initially detected as a single population with specific affinity for NPY. However, the present data strongly suggest that these differently located NPY receptor sites are of two distinct subpopulations. The receptors involved in the modulatory action of NPY on VIP-induced relaxation would be similar to those encountered in brain and other peripheral tissues where deletion of Tyr¹ also resulted in the formation of a peptide with an intrinsic activity similar to that of NPY (Rioux *et al.*, 1986; Martel *et al.*, 1986; Donoso *et al.*, 1988; Dumont *et al.*, 1992). In contrast, the receptors mediating the inhibitory action of NPY on NA-evoked responses would belong to a possible new subpopulation.

In conclusion, this study shows that NPY receptors in guinea-pig trachea are far from being homogeneous. That NPY(2-36) expresses antagonistic property specifically on one NPY receptor subpopulation exemplifies the diverse roles of NPY fragments as biologically active molecules.

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