

Effect of neuropeptide Y on human bronchus and its modulation of neutral endopeptidase

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Background: Neuropeptide Y (NPY) and neutral endopeptidase have been shown to regulate the response of airway smooth muscle. However, their interactions have not been studied.

Methods: To determine the role of NPY and endogenous neutral endopeptidase in regulating the effects of NPY, we investigated the effects of NPY and a neutral endopeptidase inhibitor, phosphoramidon, on the contractile response of isolated human bronchial segments to electrical field stimulation (EFS).

Results: Concentrations of up to 10^{-6} mol/L of NPY did not change resting tension. NPY inhibited the cholinergic contractions induced by EFS at 5 Hz dose-dependently. Phosphoramidon (10^{-5} mol/L) enhanced the inhibitory effect of NPY on EFS-induced contractions at 5 Hz. NPY (3×10^{-7} mol/L) shifted the frequency-response curve for EFS (1 to 50 Hz) to the right and significantly increased the mean logarithm of frequency that produced 50% of maximal contraction (control: 0.54 ± 0.07 versus NPY: 0.79 ± 0.09 , $p < 0.05$). Phosphoramidon (10^{-5} mol/L) did not affect the frequency response to EFS, but enhanced the inhibitory effect of NPY (3×10^{-7} mol/L) on frequency response to EFS, resulting in a significant increase in the mean logarithm of frequency that produced 50% of maximal contraction (NPY: 0.79 ± 0.09 versus NPY plus phosphoramidon: 1.18 ± 0.15 , $p < 0.05$). NPY (3×10^{-7} mol/L) did not affect the response of tissues to exogenously applied acetylcholine.

Conclusions: These observations suggest that NPY inhibits the output of acetylcholine from nerve endings and that endogenous neutral endopeptidase modulates the effect of NPY in the human airway by inactivating this peptide. (*J ALLERGY CLIN IMMUNOL* 1993;92:89-94.)

Key words: Acetylcholine release, electrical field stimulation, human bronchus, neutral endopeptidase, neuropeptide Y

Neuropeptide Y (NPY) is a 36-amino acid peptide that is present in the central and peripheral nervous systems.¹⁻³ In vascular smooth muscle, this agent induces vasoconstriction, not only enhancing noradrenaline-induced vasoconstriction at the postjunctional level, but also suppressing the release of noradrenaline from sympathetic nerve endings at the prejunctional level.^{4, 5} In airway smooth muscle of the guinea pig, NPY has little effect on smooth muscle tone, but inhibits cholinergic contractions^{6, 7} and nonadrenergic-noncholinergic contractions at the prejunctional level.⁸⁻¹⁰ NPY is present in the bronchial vessels,

Abbreviations used

EFS:	Electrical field stimulation
Log(EF ₅₀):	Logarithm of frequency of EFS that produced 50% of maximal response
NPY:	Neuropeptide Y
NEP:	Neutral endopeptidase

mucous glands, and airway smooth muscles of animals^{11, 12} and humans.^{13, 14} Therefore it is possible that it may exert a similar inhibitory effect on the cholinergic tone of the human bronchus. However, such studies have not been reported.

Several investigations have shown that neutral endopeptidase (NEP), a membrane-bound enzyme that is located on the surfaces of many cells in the airway,¹⁵ degrades the airway tachykinins and modulates the response to these peptides in experimental animals^{16, 17} and humans.^{18, 19} A decrease in NEP activity is produced by airway inflammation, which increases the bronchomotor

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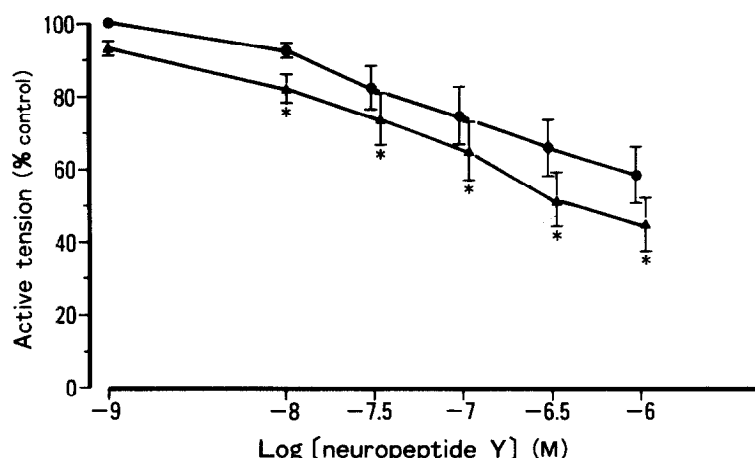


FIG. 2. Effect of increasing concentrations of NPY on contractile response to EFS at 5 Hz in presence (triangles) or absence (dots) of phosphoramidon (10^{-5} mol/L). Results are expressed as percentage of control responses before addition of NPY. Values represent mean $\pm$ SE in five strips from five subjects. Asterisk indicates $p < 0.05$ versus NPY alone.

elicited by EFS (1 to 50 Hz) were measured in paired tissues. After completion of the first frequency response elicited by EFS, tissues were incubated with either NPY (3×10^{-7} mol/L) alone or NPY (3×10^{-7} mol/L) plus phosphoramidon (10^{-5} mol/L) for 20 minutes. Then EFS (1 to 50 Hz) was applied, and a second frequency-response curve was obtained.

Effect of NPY on dose-response curves to acetylcholine

To determine whether the effects of NPY were mediated by postsynaptic mechanisms, we assessed the effects of NPY (3×10^{-7} mol/L) on the response to acetylcholine (10^{-8} mol/L to 10^{-3} mol/L). After the first dose-response curve to acetylcholine was obtained, the tissues were washed repeatedly and then incubated with NPY (3×10^{-7} mol/L) for 20 minutes. A second dose-response curve to acetylcholine was then obtained.

Drugs

The following agents were used: acetylcholine chloride, atropine sulfate, diphenhydramine, hexamethonium bromide, indomethacin, propranolol, and tetrodotoxin (Sigma, St. Louis, Mo.); phentolamine mesylate (Ciba-Geigy, Takarazuka, Japan); and NPY and phosphoramidon (Peptide Institute, Osaka, Japan). These agents were dissolved in distilled water and diluted with Krebs solution for each experiment. The molar concentrations of agents described in this paper refer to the final bath concentrations.

Data analysis

Values are mean $\pm$ SE; n indicates the number of bronchial preparations evaluated. The logarithm of frequency of EFS that produced 50% of maximal response was designated as the $\log(\text{EF}_{50})$. Statistical analyses were performed by Student's paired and un-

paired t tests as appropriate. A p value of less than 0.05 was considered to be statistically significant.

RESULTS

Characteristics of response to EFS

EFS evoked frequency-dependent rapid contractions at resting tension. Atropine (10^{-6} mol/L) and tetrodotoxin (10^{-6} mol/L) abolished contractile responses to EFS from 1 to 50 Hz. The ganglionic blocker hexamethonium (10^{-5} mol/L) had no significant effects ($p > 0.5$; $n = 3$).

Dose-response effects of NPY on EFS-induced contractions at 5 Hz

Concentrations of up to 10^{-6} mol/L of NPY had no significant effect on resting tension ($p > 0.5$; $n = 5$; Fig. 1). However, NPY inhibited contractile responses to EFS at 5 Hz in a dose-dependent manner (Figs. 1 and 2). Phosphoramidon (10^{-5} mol/L) significantly enhanced the inhibitory effects of NPY at concentrations of 10^{-8} mol/L to 10^{-6} mol/L on EFS-induced contractions (Fig. 2).

Effect of NPY on frequency-response curves for EFS

NPY (3×10^{-7} mol/L) shifted the mean frequency-response curve for EFS to the right (Fig. 3) and was associated with significant increases in the $\log(\text{EF}_{50})$ values (Table 1). Phosphoramidon (10^{-5} mol/L) had no independent effect on the EFS frequency-response curve (Fig. 4). However, phosphoramidon (10^{-5} mol/L) enhanced the inhibitory effect of NPY (3×10^{-7} mol/L) on the EFS frequency-response curve and shifted the mean frequency-response curve to the right (Fig.

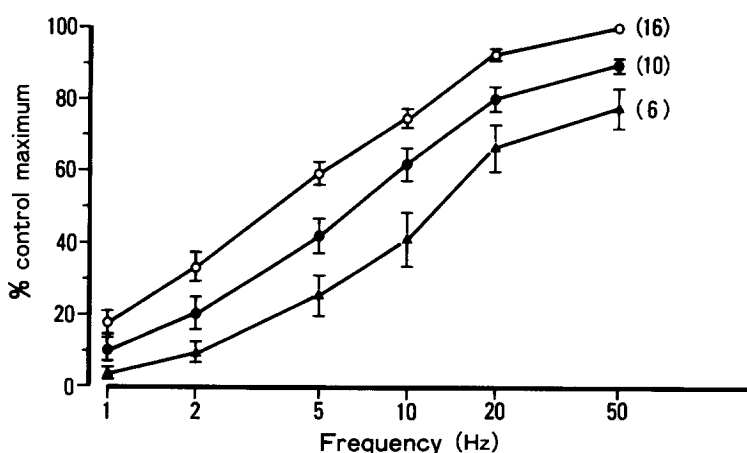


FIG. 3. Effect of NPY (3×10^{-7} mol/L) on EFS frequency-response curve in presence (triangles) or absence (solid dots) of phosphoramidon (10^{-5} mol/L) compared with control response curve (open dots). Results are expressed as percentage of initial control responses to EFS at 50 Hz. Values represent mean $\pm$ SE of number of preparations given in parentheses. Bronchial segments were obtained from six subjects.

TABLE I. Effect of NPY and phosphoramidon on EFS frequency-response curve

	Log (EF ₅₀)	n
Control	0.54 $\pm$ 0.07	16
NPY alone	0.79 $\pm$ 0.09	10*
NPY plus phosphoramidon	1.18 $\pm$ 0.15	6†

Values are means $\pm$ SE and are expressed as mean log (EF₅₀) to EFS.

* $p < 0.05$.

† $p < 0.001$ versus control; $p < 0.05$ versus NPY alone.

3), as the increased log(EF₅₀) values demonstrate (Table I). The inhibitory effect of NPY on the contractile response to EFS was more pronounced at low frequencies than at high frequencies (Fig. 4).

Effect of NPY on dose-response curves for acetylcholine

In contrast to the EFS-induced contractions, the response to doses of acetylcholine ranging from 10^{-8} to 10^{-3} mol/L was not affected by the addition of 3×10^{-7} mol/L of NPY (Fig. 5). Phosphoramidon (10^{-5} mol/L) had no effect on the response to acetylcholine (data not shown; $p > 0.5$; $n = 4$).

DISCUSSION

Our results demonstrated that NPY inhibited the EFS-induced contractions of human bronchi via presynaptic mechanisms and that these inhibitory effects were modulated by endogenous NEP.

The issue of whether the human airway smooth muscle is innervated by sympathetic fibers is still being debated. Some studies have demonstrated a

minimal adrenergic innervation,²⁶ whereas others have denied its presence.²⁷ Therefore sympathetic nerve activity is believed not to play a major role in the human airway. However, immunohistochemical studies have identified the presence of nerve fibers containing immunoreactive NPY in bronchial smooth muscle layers in animals^{11, 12} and in humans.^{13, 14} NPY has also been detected in the blood,²⁸ which suggests that it may reach nerve endings from the bloodstream. NPY may therefore play a role in regulating bronchomotor tone.

Several experiments have shown that EFS induces rapid cholinergic contractions followed by nonadrenergic-noncholinergic relaxations in human airways.²⁹ We observed cholinergic contractions in all bronchial samples, but nonadrenergic-noncholinergic relaxations were either absent or very mild at the resting tension. As shown in previous studies,²⁹ initial contraction can be blocked by atropine or tetrodotoxin, which confirms that these contractions are mediated via acetylcholine released from postganglionic cholinergic nerve endings.

Several studies have shown that NPY suppressed not only cholinergic neurotransmission but also neurotransmission in nonadrenergic-noncholinergic excitatory nerve in the guinea pig airway.⁶⁻¹⁰ Our study demonstrated that NPY had no effect in human bronchi on basal tension, but that it inhibited the EFS-induced contractions dose-dependently. Our study also demonstrated that this inhibition was pronounced at low frequencies. NPY did not affect the contractions induced by exogenously applied acetylcholine. Therefore, the inhibitory effect of NPY was me-

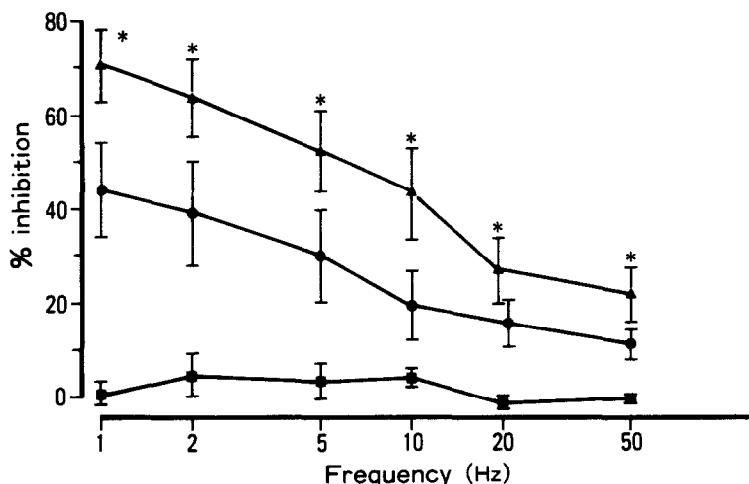


FIG. 4. Inhibitory effect of NPY (3×10^{-7} mol/L) on frequency-response curve for EFS in the presence (triangles) and absence (circles) of phosphoramidon (10^{-5} mol/L) compared with response incubated with phosphoramidon alone (squares). Results are expressed as percentage of initial EFS response at each frequency before addition of NPY. Asterisks indicate $p < 0.05$ versus NPY alone.

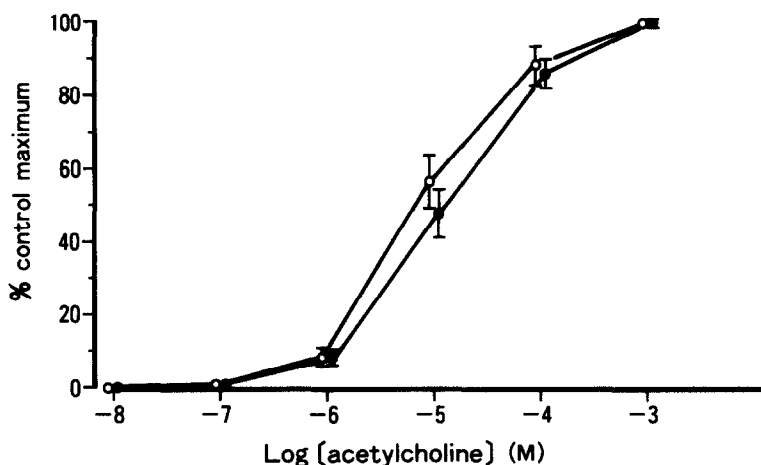


FIG. 5. Dose-response curve for acetylcholine before (open dots) and after (solid dots) addition of NPY (3×10^{-7} mol/L). Results are expressed as percentage of initial maximal contractions induced by acetylcholine before addition of NPY. Values represent mean $\pm$ SE in eight strips from four subjects.

diated by a presynaptic mechanism, as demonstrated by previous experiments in guinea pig trachea.^{6, 7, 10} Because the tissues were maintained in a medium that contained phentolamine and propranolol throughout the experiment, NPY-induced changes in sympathetic nerve activity were unlikely to be involved.

Several proteases including NEP play important roles in regulating the effects of several neuropeptides in the human airway.^{18, 19, 30} Viral infection of the airway can reduce the activity of NEP and increase the bronchial response to tachykinins.^{20, 21} NEP has also been shown to de-

grade NPY in human nasal mucosa.²⁴ Our study demonstrated that phosphoramidon enhanced the inhibitory effect of NPY on the EFS-induced contractions in the human bronchus, which suggests that endogenous NEP can degrade NPY, modulating its effect. This observation suggests that NPY may act strongly in the inflamed airways and maintain a balance of bronchomotor tone. However, phosphoramidon alone had no effect on EFS-induced contractions, which indicates that endogenous neuropeptides, including NPY, did not play a major role.

It is known that NEP can be inactivated by

cigarette smoking.³¹ Most of the tissues in this study were obtained from patients with a history of smoking. If cigarette smoking had reduced the NEP activity in these tissues, we may have underestimated the role of endogenous NEP. Results may also differ in patients with asthma; no samples from patients with asthma were included in this study.

In conclusion, we demonstrated that NPY inhibited the parasympathetic response in human bronchial segments and that this effect was regulated by endogenous NEP. Thus NPY, as well as vasoactive intestinal polypeptides, may exert an important role in braking the effect of tachykinins on the human airway.

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REFERENCES

- O'Hare MMT, Tenmoku S, Aakerlund L, Hislsted L, Johnsen A, Schwartz TW. Neuropeptide Y in guinea pig, rabbit, rat and man: identical amino acid sequence and oxidation of methionine-17. *Regul Pept* 1988;20:293-304.
- Adrian TE, Allen JM, Bloom SR, et al. Neuropeptide Y distribution in human brain. *Nature* 1983;306:584-6.
- Polak JM, Bloom SR. Regulatory peptide—the distribution of two newly discovered peptides: PHI and NPY. *Peptides* 1984;5:79-89.
- Pernow J, Saria A, Lundberg JM. Mechanisms underlying pre- and postjunctional effects of NPY in sympathetic vascular control. *Acta Physiol Scand* 1986;126:239-49.
- Wahlestedt C, Yanaihara N, Hakanson R. Evidence for different pre- and post-junctional receptors for neuropeptide Y and related peptides. *Regul Pept* 1986;13:307-18.
- Stretton CD, Barnes PJ. Modulation of cholinergic neurotransmission in guinea-pig trachea by neuropeptide Y. *Br J Pharmacol* 1988;93:672-8.
- Grundemar L, Widmark E, Waldeck B, Hakanson R. Neuropeptide Y: prejunctional inhibition of vagally induced contractions in the guinea pig trachea. *Regul Pept* 1988;23:309-13.
- Matran R, Martling C-R, Lundberg JM. Inhibition of cholinergic and non-adrenergic, non-cholinergic bronchoconstriction in the guinea pig mediated by neuropeptide Y and α_2 -adrenoceptors and opiate receptors. *Eur J Pharmacol* 1989;163:15-23.
- Giuliani S, Maggi CA, Meli A. Prejunctional modulatory action of neuropeptide Y on peripheral terminals of capsaicin-sensitive sensory nerves. *Br J Pharmacol* 1989;98:407-12.
- Grundemar L, Grundström N, Johansson IGM, Andersson RGG, Hakanson R. Suppression by neuropeptide Y of capsaicin-sensitive sensory nerve-mediated contraction in guinea pig airways. *Br J Pharmacol* 1990;99:473-6.
- Uddman R, Sundler F, Emson P. Occurrence and distribution of neuropeptide Y immunoreactive nerves in the respiratory tract and middle ear. *Cell Tissue Res* 1984;237:321-7.
- Martling CR, Matran R, Alving K, Hökfelt T, Lundberg JM. Innervation of lower airways and neuropeptide effects on bronchial and vascular tone in the pig. *Cell Tissue Res* 1990;260:223-33.
- Sheppard MN, Polak JM, Allen JM, Bloom SR. Neuropeptide tyrosine (NPY): a newly discovered peptide is present in the mammalian respiratory tract. *Thorax* 1984;39:326-30.
- Björklund A, Hökfelt T, Owman C. Airway innervation. In: Björklund A, Hökfelt T, eds. *Handbook of chemical neuroanatomy: the peripheral nervous system*. Amsterdam: Elsevier Science, 1988:391-444.
- Johnson AR, Ashton J, Schulz WW, Erdos EG. Neutral metalloendopeptidase in human lung tissue and cultured cells. *Am Rev Respir Dis* 1985;132:564-8.
- Sekizawa K, Tamaoki J, Nadel FA, Borson DB. Enkephalinase inhibitor potentiates substance P- and electrically induced contraction in ferret trachea. *J Appl Physiol* 1987;63:1401-5.
- Stimler-Gerard NP. Neutral endopeptidase-like enzyme controls the contractile activity of substance P in guinea-pig lung. *J Clin Invest* 1987;79:1819-25.
- Honda I, Khorogi H, Yamaguchi T, Ando M, Araki S. Enkephalinase inhibitor potentiates substance P- and capsaicin-induced bronchial smooth muscle contractions in humans. *Am Rev Respir Dis* 1991;143:1416-8.
- Frossard N, Barnes PJ. Effect of tachykinins in small human airway. *Neuropeptides* 1991;19:157-61.
- Jacoby DB, Tamaoki J, Borson DB, Nadel JA. Influenza infection causes airway hyperresponsiveness by decreasing enkephalinase. *J Appl Physiol* 1988;64:2653-8.
- Dusser DJ, Jacoby DB, Djokic TD, Rubinstein I, Borson DB, Nadel JA. Virus induces airway hyperresponsiveness to tachykinins: role of neutral endopeptidase. *J Appl Physiol* 1989;67:1504-11.
- Widdicombe JG. Neural control of airway vasculature and edema. *Am Rev Respir Dis* 1991;143:18-21.
- Nadel JA. Neutral endopeptidase modulates neurogenic inflammation. *Eur Respir J* 1991;4:745-54.
- Baraniuk JN, Castellino S, Lundgren JD, et al. Neuropeptide Y (NPY) in human nasal mucosa. *Am J Respir Cell Mol Biol* 1990;3:165-73.
- Hudgin RL, Charleson SE, Zimmerman M, Mumford R, Wood PL. Enkephalinase: selective peptide inhibitors. *Life Sci* 1981;29:2593-601.
- Laitinen A, Partanen M, Hervonen A, Laitinen LA. Electron microscopic study on the innervation of human lower respiratory tract: evidence of adrenergic nerves. *Eur Respir J* 1985;67:209-15.
- Doidge JM, Satchell DG. Adrenergic and non-adrenergic inhibitory nerve in mammalian airways. *J Auton Nerv Syst* 1982;5:83-9.
- Morris MS, Elliott JM, Cain MD, Kapoor V, West MJ, Chalmers JP. Plasma neuropeptide Y levels rise in patients undergoing exercise tests for the investigation of chest pain. *Clin Exp Pharmacol Physiol* 1986;13:437-40.
- Rhoden KJ, Meldrum LA, Barnes PJ. Inhibition of cholinergic neurotransmission in human airways by β -adrenoceptors. *J Appl Physiol* 1988;65:700-5.
- Tam EK, Franconi GM, Nadel JA, Caughey GH. Protease inhibitors potentiate smooth muscle relaxation induced by vasoactive intestinal peptide in isolated human bronchi. *Am J Respir Cell Mol Biol* 1990;2:449-52.
- Dusser DJ, Djokic TD, Borson DB, Nadel JA. Cigarette smoke induces bronchoconstrictor hyperresponsiveness to substance P and inactivates airway neutral endopeptidase in guinea-pig: possible role of free radicals. *J Clin Invest* 1989;84:900-6.