Effect of neuropeptide Y on α_2 -adrenoceptor-mediated cardiovascular responses in the pithed rat

¹E. Vila & *I.M. Macrae

Department de Farmacologia i Psiquiatria, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain and *University Department of Medicine and Therapeutics, Gardiner Institute, Western Infirmary, Glasgow, G11 6NT

- 1 The effects of neuropeptide Y (NPY) on the cardiovascular responses induced by stimulation of pre- and postjunctional α_2 -adrenoceptors were studied in the pithed normotensive rat.
- 2 The increase in diastolic blood pressure induced by cumulative injection of xylazine ($1\text{--}1000\ \mu\text{g kg}^{-1}$, i.v.) were potentiated by NPY ($5\ \mu\text{g kg}^{-1}$) but not affected by a lower dose ($0.75\ \mu\text{g kg}^{-1}$) of this peptide.
- 3 Xylazine ($1\text{--}100\ \mu\text{g kg}^{-1}$) inhibited in a dose-dependent manner the tachycardia induced by continuous electrical stimulation (0.2 Hz, 2 ms, 60 V) of the spinal cord (C7-Th1).
- 4 NPY ($5\ \mu\text{g kg}^{-1}$ but not $0.75\ \mu\text{g kg}^{-1}$) enhanced the inhibitory effect of xylazine on the tachycardia induced by electrical stimulation without having any direct effect on heart rate.
- 5 These results suggest that there may be a positive interaction between NPY receptors, postjunctional α_2 -adrenoceptors and between NPY receptors and prejunctional α_2 -adrenoceptors in the cardiovascular system of the rat.

Introduction

Neuropeptide Y (NPY) is a 36 amino acid peptide isolated from porcine brain (Tatemoto, 1982) which is widely distributed throughout the central and peripheral nervous systems. It co-exists with noradrenaline in many sympathetic nerves supplying the cardiovascular system and is co-released with noradrenaline by sympathetic nerve stimulation (for review see: Edvinsson *et al.*, 1987). Several *in vitro* and *in vivo* studies have demonstrated that NPY exerts a direct postjunctional action manifested as vasoconstriction which is not attenuated by α -adrenoceptor antagonists (for review see: Potter, 1988).

In the rat, as in other species, NPY has been shown to enhance the vasoconstriction caused *in vitro* by electrical stimulation or by agonists such as noradrenaline (Lundberg *et al.*, 1985; Pernow *et al.*, 1986; Westfall *et al.*, 1987). A few studies have found a potentiating effect on the pressor responses induced by α_1 -adrenoceptor agonists in both the pithed (Dahlöf *et al.*, 1985; Zukowska-Grojec *et al.*, 1986) and anaesthetized rat (Revington *et al.*, 1987). Some of these studies were carried out with noradrenaline as the α -adrenoceptor agonist. It is well known that noradrenaline stimulates α_1 - as well as α_2 -adrenoceptors (Starke, 1981). In addition, it is now very well established that many blood vessels contain both subtypes of postjunctional α -adrenoceptors. Studies carried out in the pithed rat clearly demonstrate that stimulation of both α_1 - and α_2 -adrenoceptors postjunctionally produces an increase in blood pressure (McGrath, 1982; Timmermans *et al.*, 1982).

The aim of the present study was to examine the effect of NPY on the cardiovascular responses induced by stimulation of pre- and postjunctional α_2 -adrenoceptors. The influence of NPY on blood pressure increases induced by the selective α_2 -adrenoceptor agonist, xylazine (Docherty & McGrath, 1980), as well as on the inhibition by xylazine of electrical stimulation-induced tachycardia have been examined in the pithed normotensive rat.

Methods

Male Sprague-Dawley rats (280–300 g) received atropine ($1\ \text{mg kg}^{-1}$, i.p.) to prevent muscarinic activation; 15 min later they were pithed under diethyl ether anaesthesia by inserting a blunt steel rod (diameter 1.5 mm) through the orbit and

foramen magnum down into the spinal canal (Shipley & Tilden, 1947). The trachea was cannulated and the animals were ventilated with room air ($60\ \text{strokes min}^{-1}$, $20\ \text{ml kg}^{-1}$ body wt). The left jugular vein was cannulated for drug administration and arterial blood pressure was measured by means of a Statham pressure transducer connected to a cannula placed in the right carotid artery. Heart rate was derived from the arterial pulse and both parameters were displayed on a Hewlett Packard Model 7785A recorder. Bilateral adrenalectomy and vagotomy were then performed. The rats were allowed to stabilize for 15 min before the experiment was begun. The electrically stimulated group of animals received (+)-tubocurarine ($1\ \text{mg kg}^{-1}$, i.v.) to prevent muscle contraction. In all experiments, with the exception of those where a decrease in heart rate was studied, the rats received propranolol ($1\ \text{mg kg}^{-1}$) to block β -adrenoceptor-mediated responses.

Two series of experiments were carried out. Firstly, a study was made of the diastolic blood pressure increases elicited by cumulative administration of xylazine ($1\text{--}1000\ \mu\text{g kg}^{-1}$, i.v.); dose-response curves for xylazine were determined before and 8 min after NPY (0.75 and $5\ \mu\text{g kg}^{-1}$) or its vehicle, 0.1% bovine serum albumin (BSA) in saline.

In the second set of experiments the inhibition by cumulative doses of xylazine ($1\text{--}1000\ \mu\text{g kg}^{-1}$, i.v.) of the heart rate increase induced by continuous electrical stimulation (0.2 Hz, 2 ms, 60 V) at the C7-Th1 level (Gillespie *et al.*, 1970) was studied. NPY (0.75 and $5\ \mu\text{g kg}^{-1}$) or its vehicle were injected after the tachycardia induced by electrical stimulation had stabilized and 8 min before the injection of xylazine.

The effects of xylazine after either 0.1% BSA or different doses of NPY (0.75 and $5\ \mu\text{g kg}^{-1}$) were determined in separate animals. In each study NPY was administered as a slow intravenous injection over approximately 1 min. As the doses of NPY used produced a direct vasoconstrictor effect, the injection of xylazine was always made 8 min after NPY to allow blood pressure to return to control levels.

It has been reported that NPY has a half-life of approximately 20 min in plasma after intravenous injection (Potter, 1987). Thus, we assumed that plasma levels of NPY remained elevated even after the blood pressure had returned to control levels (Revington *et al.*, 1987).

Statistical analysis

Values are expressed as mean $\pm$ s.e.mean. The statistical significance between groups was assessed by repeated measures

¹ Author for correspondence.

Figure 1 Increases in diastolic blood pressure elicited by cumulative doses of xylazine (1–1000 $\mu\text{g kg}^{-1}$) administered 8 min after vehicle ($\circ$, $n = 10$) or neuropeptide Y (NPY) 0.75 $\mu\text{g kg}^{-1}$ ($\blacktriangle$, $n = 4$) and 5 $\mu\text{g kg}^{-1}$ ($\blacksquare$, $n = 10$). Points represent the mean and the vertical bars show s.e.mean. *Significant difference from saline control value ($P < 0.05$).

analysis of variance with a Bonferroni correction for multiple comparisons. A value of $P < 0.05$ was taken as significant.

Drugs

Atropine sulphate (Merck), diethyl ether (Panreac), propranolol HCl (Sigma), (+)-tubocurarine (Sigma), xylazine HCl (Bayer), and synthetic porcine NPY (Sigma) were used. All drugs were dissolved in distilled water or saline and injected in a volume of 0.5 ml kg^{-1} body weight. NPY was initially dissolved in 0.1% BSA in saline, aliquoted into silicone-coated tubes and stored at -70°C as a stock solution (10 mg ml $^{-1}$). This was further diluted with 0.1% BSA in saline immediately before use.

Results

The resting diastolic blood pressure of normotensive pithed rats was 49 ± 1.2 mmHg, $n = 51$. Cumulative administration

Figure 2 The influence of neuropeptide Y (NPY) on the dose-response curve for the inhibitory effect of xylazine on the tachycardia induced by electrical stimulation in pithed normotensive rats. Xylazine was administered cumulatively 8 min after i.v. vehicle ($\circ$, $n = 10$) or NPY 0.75 $\mu\text{g kg}^{-1}$ ($\blacktriangle$, $n = 4$) and 5 $\mu\text{g kg}^{-1}$ ($\blacksquare$, $n = 10$). Parameters for electrical stimulation were 0.2 Hz, 2 ms, 60 V. Points represent the mean and the vertical bars show s.e.mean. *Significant difference from saline control value ($P < 0.05$).

of xylazine (1 to 1000 $\mu\text{g kg}^{-1}$) to control rats caused a dose-dependent increase in diastolic blood pressure (Figure 1). Injection of the vehicle induced a transient increase in diastolic blood pressure (5 mmHg) in some rats with a return to basal levels within 2–3 min. Systemic administration of NPY (0.75 and 5 $\mu\text{g kg}^{-1}$) produced a dose-dependent increase in diastolic blood pressure in the pithed rats (21 ± 2.3 , $n = 8$ and 54.2 ± 3.9 mmHg, $n = 20$, respectively). These increases returned to basal levels within 5–7 min. The lower dose of NPY did not modify the xylazine-induced diastolic blood pressure increases (Figure 1). In contrast, the dose-response curve for the α_2 -adrenoceptor agonist was shifted to the left after injection of 5 $\mu\text{g kg}^{-1}$ NPY. The enhancement of pressor responses to the highest doses of xylazine (300 and 1000 $\mu\text{g kg}^{-1}$) by NPY (5 $\mu\text{g kg}^{-1}$) did not reach statistical significance.

The resting heart rate of pithed rats was 409 ± 7 beats min^{-1} , $n = 51$. Continuous electrical stimulation (0.2 Hz, 2 ms, 60 V) of the spinal cord (C7-Th1) produced an increase in heart rate of 103 ± 10 beats min^{-1} ($n = 24$) that remained constant for more than 1 h. Injection of cumulative doses of xylazine (1–1000 $\mu\text{g kg}^{-1}$) produced a dose-dependent decrease in the stimulation-induced tachycardia (Figure 2). Neither NPY nor its vehicle had any effect on the tachycardia obtained in response to electrical stimulation. Nevertheless, the time between administration of BSA or NPY and the start of xylazine administration was maintained at 8 min to allow the blood pressure to return to the basal value. Eight minutes after the administration of 5 $\mu\text{g kg}^{-1}$, but not 0.75 $\mu\text{g kg}^{-1}$, NPY (Figure 2) the heart rate decreases induced by xylazine were significantly greater than those obtained in the absence of NPY, except with the lowest doses (1–10 $\mu\text{g kg}^{-1}$) where the difference did not reach statistical significance.

Discussion

Several authors have obtained potentiation of α -adrenoceptor-mediated vascular smooth muscle contraction by NPY *in vivo* and *in vitro* (Dahlöf *et al.*, 1985; Pernow *et al.*, 1986; Zukowska-Grojec *et al.*, 1986; Westfall *et al.*, 1987). Some of these studies have been carried out with the α_1 -adrenoceptor agonist phenylephrine but others have used noradrenaline, a non-selective α -adrenoceptor agonist.

Our experiments were carried out in the pithed rat which is devoid of central cardiovascular reflexes and has been proved to be a suitable model for studying the effects of drugs on both pre- and postjunctional α -adrenoceptors in the periphery. The results obtained in this study with xylazine, a selective α_2 -adrenoceptor agonist, demonstrate that NPY potentiates the pressor responses mediated by postjunctional α_2 -adrenoceptors and the prejunctional inhibition by xylazine of the stimulation-induced tachycardia.

NPY potentiates the pressor responses mediated by postjunctional α_2 -adrenoceptors suggesting a positive interaction between postjunctional α_2 -adrenoceptors and NPY receptors. Since both NPY (MacLean & Hiley, 1990) and xylazine (Hiley & Thomas, 1987) can increase cardiac output as well as total peripheral resistance, it is not possible to distinguish whether this potentiation of pressor responses involves a receptor interaction in the heart or blood vessels. NPY-induced potentiation of the pressor response was only observed with the lower doses of xylazine (up to and including 100 $\mu\text{g kg}^{-1}$), suggesting that the pressor response to xylazine was maximal at the highest doses. These results are in agreement with those observed in the rabbit ear artery (mainly α_1 -adrenoceptors) and in the canine saphenous vein (mainly α_2 -adrenoceptors; Hieble *et al.*, 1989) where NPY has a selective effect on α_2 -adrenoceptor-mediated vasoconstriction induced by low doses of noradrenaline. Zukowska-Grojec *et al.* (1986) demonstrated that, in the pithed rat, only a very low dose of NPY (0.43 $\mu\text{g kg}^{-1}$) potentiated the noradrenaline pressor responses, a higher dose (2.5 $\mu\text{g kg}^{-1}$) being without effect.

These results disagree with ours since we could not demonstrate potentiation with a low dose of NPY ($0.75 \mu\text{g kg}^{-1}$) but a clear enhancement was seen with $5 \mu\text{g kg}^{-1}$. An intermediate dose of NPY ($2.5 \mu\text{g kg}^{-1}$) produced a similar response to that of $5 \mu\text{g kg}^{-1}$ (results not shown). We have no explanation for the differences between these results other than the use of a different pressor agonist. However, both Dahlöf *et al.* (1985), in the pithed spontaneously hypertensive rat, and Revington *et al.* (1987) in anaesthetized rats, demonstrated postjunctional α -adrenoceptor potentiation of the phenylephrine-induced pressor response with higher doses of NPY (10 and $20 \mu\text{g kg}^{-1}$). A more recent study in conscious rats (Aubert *et al.*, 1988) demonstrated a potentiation by NPY of noradrenaline-induced pressor responses but failed to show NPY potentiation of the increases in diastolic blood pressure induced by the α_2 -adrenoceptor selective agonist BHT-933. The difference between this and the present study may be explained by the use of different agonists and animal models.

Experiments in which NPY has been shown to enhance the release of [^3H]-noradrenaline have led to the suggestion that NPY could act on prejunctional NPY receptors modulating the release of noradrenaline (Edvinsson *et al.*, 1987). Zukowska-Grojec *et al.* (1986) have shown that NPY ($0.43 \mu\text{g kg}^{-1}$) attenuates electrically-induced tachycardia without modifying the neuronal release of noradrenaline. In contrast, Dahlöf *et al.* (1988) have shown that NPY causes an

increase in heart rate and a reduction in noradrenaline release following electrically-induced stimulation of the spinal cord. Both studies were carried out in the pithed rat. In the present study we were unable to demonstrate any modification by NPY of the stimulation-induced tachycardia, but a significant enhancement of the inhibitory effect of xylazine was observed. These results could be interpreted as NPY producing prejunctional potentiation of the α_2 -adrenoceptor stimulation and may represent a positive interaction between NPY receptors and prejunctional α_2 -adrenoceptors in the heart. A postjunctional inhibition of β_1 -adrenoceptors is unlikely since NPY did not influence heart rate in the absence of xylazine.

The fact that NPY has been found to increase the accumulation of inositol phosphate by phenylephrine in the rat vas deferens (Hagglad & Fredholm, 1987) suggests that the potentiating effect of NPY on α_1 -adrenoceptor-mediated responses could be mediated via the inositol pathway. However, it has also been shown that NPY inhibits adenylate cyclase through a pertussis toxin-sensitive mechanism (Fredholm *et al.*, 1985; Lundberg *et al.*, 1988). Since it is known that activation of α_2 -adrenoceptors produces inhibition of adenylate cyclase (Exton, 1985), this could be the common mechanism for activation and potentiation of α_2 -adrenoceptor-mediated responses.

We would like to thank Bayer (Spain) for the gift of xylazine HCl.

References

- AUBERT, J.F., WAEBER, B., ROSSIER, B., GEERING, K., NUSSBERGER, J. & BRUNNER, H.R. (1988). Effects of neuropeptide Y on the blood pressure response to various vasoconstrictor agents. *J. Pharmacol. Exp. Ther.*, **246**, 1088–1092.
- DAHLÖF, C., DAHLÖF, P. & LUNDBERG, J.M. (1985). Neuropeptide Y (NPY): enhancement of blood pressure increase upon α -adrenoceptor activation and direct pressor effects in pithed rats. *Eur. J. Pharmacol.*, **109**, 289–292.
- DAHLÖF, P., PERSSON, K., LUNDBERG, J.M. & DAHLÖF, C. (1988). Neuropeptide Y (NPY) induced inhibition of preganglionic nerve stimulation evoked release of adrenaline and noradrenaline in the pithed rat. *Acta Physiol. Scand.*, **132**, 51–57.
- DOCHERTY, J.R. & McGRATH, J.C. (1980). A comparison of pre- and postjunctional potencies of several α -adrenoceptor agonists in the cardiovascular system and anococcygeus muscle of the rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **312**, 107–116.
- EDVINSSON, L., HAKANSON, R., WAHLESTEDT, C. & UDDMAN, R. (1987). Effect of neuropeptide Y on the cardiovascular system. *Trends Pharmacol. Sci.*, **8**, 231–235.
- EXTON, J.H. (1985). Mechanisms involved in α -adrenergic phenomena. *Am. J. Physiol.*, **248**, E633–E677.
- FREDHOLM, B.B., JANSEN, I. & EDVINSSON, L. (1985). Neuropeptide Y is a potent inhibitor of cyclic AMP accumulation in feline cerebral blood vessels. *Acta Physiol. Scand.*, **124**, 467–469.
- GILLESPIE, J.S., MACLAREN, S. & POLLOCK, D. (1970). A method of stimulating different segments of the autonomic outflow from the spinal column to various organs in the pithed cat and rat. *Br. J. Pharmacol.*, **40**, 257–267.
- HAGGLAD, J. & FREDHOLM, B.B. (1987). Adenosine and neuropeptide Y enhance α_1 -adrenoceptor-induced accumulation of inositol phosphates and attenuate forskolin-induced accumulation of cyclic AMP in rat vas deferens. *Neurosci. Lett.*, **82**, 211–216.
- HIEBLE, J.P., DUESLER, J.G. & DALY, R.N. (1989). Effects of neuropeptide Y on the response of isolated blood vessels to norepinephrine and sympathetic field stimulation. *J. Pharmacol. Exp. Ther.*, **250**, 523–528.
- HILEY, C.R. & THOMAS, G.R. (1987). Effects of α -adrenoceptor agonists on cardiac output and its regional distribution in the pithed rat. *Br. J. Pharmacol.*, **90**, 61–70.
- LUNDBERG, J.M., PERNOW, J., TATEMOTO, K. & DAHLÖF, C. (1985). Pre- and postjunctional effect of NPY on sympathetic control of rat femoral artery. *Acta Physiol. Scand.*, **123**, 511–513.
- LUNDBERG, J.M., HEMSEN, A., LARSSON, O., RUDEHILL, A., SARIA, A. & FREDHOLM, B.B. (1988). Neuropeptide Y receptors in pig spleen: binding characteristics, reduction of cyclic AMP formation and calcium antagonist inhibition of vasoconstriction. *Eur. J. Pharmacol.*, **145**, 21–29.
- MACLEAN, M.R. & HILEY, C.R. (1990). Effect of neuropeptide Y on cardiac output, its distribution, regional blood flow and organ vascular resistances in the pithed rat. *Br. J. Pharmacol.*, **99**, 340–342.
- McGRATH, J.C. (1982). Evidence for more than one type of postjunctional α -adrenoceptor. *Biochem. Pharmacol.*, **31**, 467–484.
- PERNOW, J., SARIA, A. & LUNDBERG, J.M. (1986). Mechanisms underlying pre- and postjunctional effects of neuropeptide Y in sympathetic vascular control. *Acta Physiol. Scand.*, **126**, 239–249.
- POTTER, E.K. (1987). Cardiac and vagal actions of neuropeptide Y following intravenous injection in the dog. *Neurosci. Lett.*, **77**, 243–247.
- POTTER, E.K. (1988). Neuropeptide Y as an autonomic neurotransmitter. *Pharmacol. Ther.*, **37**, 251–273.
- REVINGTON, M.L., McCLOSKEY, D.I. & POTTER, E.K. (1987). Effects of neuropeptide Y on the pressor responses to phenylephrine and to activation of the sympathetic nervous system in anaesthetized rats. *Clin. Exp. Pharmacol. Physiol.*, **14**, 703–710.
- SHIPLEY, R. & TILDEN, J.H. (1947). A pithed rat preparation suitable for assaying pressor substances. *Proc. Soc. Exp. Biol. (NY)*, **64**, 453–455.
- STARKE, K. (1981). Alpha-adrenoceptor sub-classification. *Rev. Physiol. Biochem. Pharmacol.*, **88**, 199–236.
- TATEMOTO, K. (1982). Neuropeptide Y: Complete amino-acid sequence of the brain peptide. *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 5485–5489.
- TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1982). α_2 -Adrenoceptors: classification, localization and target for drugs. *J. Med. Chem.*, **25**, 1389–1401.
- WESTFALL, T.C., CARPENTIER, S., CHEN, X., BEINFELD, M.C., NAES, L. & MELDRUM, M.J. (1987). Prejunctional and postjunctional effects of neuropeptide Y at the noradrenergic neuroeffector junction of the perfused mesenteric arterial bed of the rat. *J. Cardiovasc. Pharmacol.*, **10**, 716–722.
- ZUKOWSKA-GROJEC, Z., HAASS, M. & BAYORH, M.A. (1986). Neuropeptide Y and peptide YY mediate non-adrenergic vasoconstriction and modulate sympathetic responses in rats. *Regul. Peptides*, **15**, 99–110.

(Received November 29, 1989
Revised March 26, 1990
Accepted March 28, 1990)