Effect of neuropeptide Y on cardiac output, its distribution, regional blood flow and organ vascular resistances in the pithed rat

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1 The effects of neuropeptide Y on cardiac output, its distribution and organ vascular resistances were determined with tracer microspheres in pithed rats.

2 Neuropeptide Y increased blood pressure by increasing both cardiac output and total peripheral resistance. The increase in cardiac output was due to an increase in stroke volume as heart rate was not changed. Increased vascular resistance in the splenic, renal, testicular, epididymal, skeletal muscle, large intestinal and mesenteric vascular beds contributed to the increase in total peripheral resistance. Vasoconstriction was most pronounced in the mesenteric bed.

3 This study indicates that neuropeptide Y increases blood pressure by increasing cardiac output and total peripheral resistance. The increased cardiac output is possibly due to an increase in venous return, whilst the increased total peripheral resistance was due to regional vasoconstriction, particularly in the mesenteric bed.

Introduction

Neuropeptide Y is a 36 amino acid peptide, originally isolated from porcine brain (Tatemoto, 1982). There is increasing evidence that this peptide is involved in the sympathetic vasoconstrictor response; it is co-stored and co-released with noradrenaline from perivascular sympathetic nerve endings and from the adrenal medulla in most species including man (Lundberg *et al.*, 1983; 1984). Low doses of neuropeptide Y, which alone do not induce vasoconstriction, have been shown to facilitate vasoconstrictor responses to noradrenaline, α -adrenoceptor agonists and sympathetic nerve stimulation in pithed and anaesthetized rats (Dahlöf *et al.*, 1985; Revington *et al.*, 1987). Higher doses of neuropeptide Y induce a direct pressor effect in pithed rats (Dahlöf *et al.*, 1985). Previous studies have been unable to ascertain whether neuropeptide Y-induced pressor responses are due to effects on cardiac output, total peripheral resistance or both. In the present study by use of tracer microspheres, we have examined the effects of neuropeptide Y on cardiac output, cardiac output distribution, total peripheral resistance and organ vascular resistance in the pithed rat.

Methods

Determination of cardiac output, its distribution and regional vascular resistance

Male Wistar rats, weighing 250–300 g (Bantin & Kingman Ltd, Hull) were pithed under halothane anaesthesia using a 16 gauge steel needle. Immediately after pithing, the rats were respired with air through a tracheal cannula by means of a respiratory pump (BioScience, Sheerness, U.K.) operating at 54 cycles min^{-1} with a volume of 20 ml kg^{-1} . The right femoral artery was cannulated and connected to a Bell & Howell type 4-422-0001 pressure transducer to measure systemic arterial blood pressure which was recorded on a Grass 7D polygraph; heart rate was determined from the systemic blood pressure trace by a Grass 7P44 cardiograph. The left femoral artery was also cannulated and connected to a

Braun Perfusor IV pump (Melsungen, F.R.G.) for the withdrawal of blood. With the aid of pressure monitoring, a cannula was passed down the right common carotid artery into the left ventricle. Rectal temperature was maintained at 37°C by means of a homeothermic blanket system (BioScience, Sheerness, Kent). Neuropeptide Y (500 pmol bolus injection (0.5 ml) followed by a 200 pmol min^{-1} infusion (0.1 ml min^{-1})) [porcine neuropeptide Y, Neosystems S.A., Strasbourg, France] or saline (0.5 ml bolus followed by an 0.1 ml min^{-1} infusion) were administered through a cannula previously placed in the left external jugular vein. Pressor responses to neuropeptide Y were always maximal and sustained 1 min after injection, and at this time (in both the neuropeptide Y-treated and saline-treated animals), 60,000–80,000 ^{113}Sn labelled microspheres ($15 \pm 3 \mu\text{m}$; NEN, Boston, MA, U.S.A.), suspended by ultrasonication in saline containing 0.01% Tween 80, were injected into the ventricle over 20 s. Blood was withdrawn from the left femoral artery at a rate of 0.5 ml min^{-1} during and, for 70 s after, the microsphere injection. The circulation was stopped with an air embolism and the organs dissected out, weighed and placed in scintillation vials for counting in a Packard Autogamma 500 γ -scintillation counter.

The number of counts in the blood sample was also determined in order that cardiac output and organ vascular resistances could be determined as described by McDevitt & Nies (1976) and by us previously (MacLean & Hiley, 1988a,b). All results are given as the mean $\pm$ s.e.mean. The statistical significance between groups was assessed by one-way random block, analysis of variance followed by the least significant difference procedure (Snedecor & Cochran, 1980).

Results

Table 1 shows that there were no significant differences in basal blood pressure or basal heart rate between the control and neuropeptide Y-treated groups. In the neuropeptide Y-treated group, the peptide increased both mean arterial and diastolic blood pressure by 78%. This was due to the combined effects of a 40% greater cardiac output and 38% greater total peripheral resistance than that of the saline-treated group. The enhanced cardiac output was due to a 34% increase in stroke volume, since heart rate was not affected by this dose of neuropeptide Y. Increased vascular resistance in the splenic, renal, testicular, epididymal, skeletal muscle,

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Table 1 Effects of neuropeptide Y (500 pmol bolus + 200 pmol min⁻¹ infusion) on blood pressure, cardiac index, heart rate and organ vascular resistance in the pithed rat as determined by use of tracer microspheres

	Saline control	Neuropeptide Y
Basal mean arterial pressure (mmHg)	36.9 ± 3.5	37.5 ± 2.5
Change in mean arterial pressure	-1 ± 1	29.4 ± 4.0***
Basal diastolic pressure (mmHg)	27.5 ± 3.5	30.6 ± 2.0
Change in diastolic pressure	-1 ± 1	24 ± 3***
Basal heart rate (beats min ⁻¹)	318 ± 9	329 ± 14
Change in heart rate	-3 ± 4	-3 ± 5
Cardiac index (ml min ⁻¹ × 100 g ⁻¹ body wt.)	11.3 ± 0.7	15.8 ± 1.0**
Stroke volume (μl)	104 ± 8	139 ± 10*
TPR (mmHg ml ⁻¹ min × 100 g body wt.)	3.13 ± 0.21	4.33 ± 0.46*
Organ vascular resistances (mmHg ml⁻¹ min g)		
Heart	23.3 ± 2.7	29.8 ± 2.6
Lungs	86.6 ± 16.0	35.2 ± 6.4**
Liver	313 ± 36	316 ± 47
Spleen	60.0 ± 5.6	89.2 ± 10.6*
Kidneys	15.7 ± 1.8	42.5 ± 6.9**
Testes	224 ± 12	327 ± 46*
Fat	3232 ± 674	6622 ± 1830
Epididimides	506 ± 45	746 ± 92*
Skeletal muscle	527 ± 71	821 ± 117*
Skin	743 ± 127	832 ± 151
Stomach	94 ± 13	103 ± 11
Small intestine	33.7 ± 2.8	45.1 ± 4.9
Large intestine	38.7 ± 6.0	69.6 ± 10.9*
Pancreas & mesentery	90.1 ± 11.1	366 ± 57***

For all groups, $n = 8$.

Significant differences between the saline (0.5 ml bolus + 0.1 ml min⁻¹ infusion) control group and the neuropeptide Y-treated group were determined by one-way analysis of variance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Basal values of heart rate, diastolic and mean arterial blood pressures were obtained before the administration of either saline or neuropeptide Y. Changes are those occurring between these values and the midpoint of the microsphere injection. Cardiac index is the absolute value determined by the tracer microsphere method. TPR is the total peripheral resistance calculated from cardiac index and mean arterial pressure during the microsphere injection assuming central venous pressure to be zero.

large intestinal and mesenteric vascular beds (49%, 171%, 46%, 47%, 56%, 79% and 306% increases respectively, compared to the saline-treated control group) contributed to the increased total peripheral resistance (Table 1).

Neuropeptide Y induced a decrease in the vascular resistance in the lungs (59% compared with the saline control group) and an increase in the fractional distribution of cardiac output to these organs (by 235% compared to the saline control group). It also induced an increase in the proportion of the cardiac output passing to the small intestine (by 17%) while causing a decrease in that passing to the kidneys and the pancreas/mesentery by 42% and 62% respectively (Figure 1).

Discussion

This study shows that neuropeptide Y exerts its pressor effect in the pithed rat by increasing both cardiac output and total peripheral resistance. The increased cardiac output was due to an increase in stroke volume since heart rate was not changed by neuropeptide Y administration. Pressor responses to neuropeptide Y which were not accompanied by bradycardia have previously been observed in the pithed rat (Dahlöf *et al.*, 1985). In the pithed rat, increased stroke volume could be due to greater venous return, an increase in cardiac contractility or both. Coronary arteries are richly innervated by neuropeptide Y-containing nerve fibres (Allen *et al.*, 1983) and neuropeptide Y has been shown to cause both coronary vasoconstriction and a reduction in cardiac contractility in isolated heart preparations and in the conscious rabbit (Minson

Figure 1 Effect of neuropeptide Y on the fractional distribution of cardiac output in pithed rats. The hatched columns represent the results from rats given saline (0.5 ml bolus + 0.1 ml min⁻¹ infusion) and the open columns are data from rats given neuropeptide Y (500 pmol bolus + 200 pmol min⁻¹ infusion). $n = 8$ in both groups. Significant differences between the saline control group and the neuropeptide Y-treated group were determined by one-way analysis of variance. ** $P < 0.01$; *** $P < 0.001$.

et al., 1987). Thus it is likely, that in the pithed rat, the increased stroke volume observed after neuropeptide Y administration is due to an increase in venous return. Increased vascular resistance in the splenic, renal, testicular, epididymal, skeletal muscle, large intestinal and mesenteric vascular beds contributed to the increase in total peripheral resistance, the increase being most profound in the mesenteric bed. This is compatible with reports that neuropeptide Y induces pressor responses in the perfused mesenteric arterial bed of the rat (Westfall *et al.*, 1987). Neuropeptide Y also induced a decrease in resistance in the lungs and a concomitant increase in the percentage of cardiac output passing to these organs. It should be noted that the microspheres detected in the lungs represent not only those passing through the bronchial arteries but also those passing through arteriovenous anastomoses and which are subsequently trapped in the lungs after passage along the great veins. Thus any effects of neuropeptide Y on lung vascular resistance could be due to actions on either the anastomoses or the bronchial arteries.

Increased renal and mesenteric vascular resistance decreased the proportion of cardiac output sent to these vascular beds and increased that sent to other organs such as the small intestine and lungs. It is of interest that neuropeptide Y caused renal vasoconstriction in this study. Plasma neuropeptide Y levels are increased after haemorrhage in conscious rats (Morris *et al.*, 1987) and in the pig (Lundberg *et al.*, 1987) and renal vasoconstriction is severe in both clinical and experimental hypovolaemic shock (Hock *et al.*, 1982; Hardaway, 1985; MacLean *et al.*, 1989b). Increased levels of circulating neuropeptide Y may, therefore, exacerbate the renal vasoconstriction observed in haemorrhagic shock.

It is becoming apparent that the pharmacological profile of neuropeptide Y has some similarity to that of the vasoactive peptide endothelin-1, recently isolated from endothelial cells in culture (Yanagisawa *et al.*, 1988). We have previously shown that endothelin-1 (300 pmol) increases total peripheral resistance mainly by vasoconstricting the mesenteric vascular bed (MacLean *et al.*, 1989a) and others have reported that more potent doses of endothelin-1 also increase vascular resistance in the kidneys and in skeletal muscle (Thomas *et al.*, 1989; Walder *et al.*, 1989). Furthermore, we have shown that

in conditions of moderate hypoxia, hypercapnia and acidosis, the potency of both endothelin-1 (MacLean *et al.*, 1989a) and neuropeptide Y (MacLean & Hiley, unpublished observations) in the blood-perfused mesenteric arterial bed of the pithed rat is increased and we have observed that both neuropeptide Y (MacLean & Hiley, unpublished observations) and endothelin-1 (MacLean *et al.*, 1989a) induce a unique fluctuation of blood pressure and heart rate in such hypoxic conditions. Neuropeptide Y-induced vasoconstriction in the rabbit ear artery is endothelium-dependent and it has been suggested that neuropeptide Y could promote the release of an endothelial derived vasoconstricting factor (Daly & Hieble, 1987). Whilst there are differences in the pharmacological profiles of neuropeptide Y and endothelin in the conscious rat (Zukowska-Grojec *et al.*, 1987; Gardiner *et al.*, 1988; 1989; Han *et al.*, 1989) it is interesting to speculate that, in some vascular beds, the activities of these two peptides may be related in some way.

It has been observed by others that in many ways, neuropeptide Y mimics the effects of noradrenaline in that it inhibits

release of noradrenaline by acting on prejunctional sites, induces vasoconstriction and bradycardia *in vivo* and contracts isolated veins and arteries *in vitro* (Dahlöf *et al.*, 1985; Pernow *et al.*, 1986). Indeed, the effects of neuropeptide Y on cardiac output, total peripheral resistance and organ vascular resistance described here are similar to those induced by α -adrenoceptor agonists as described in previous microsphere studies (Hiley & Thomas, 1987; MacLean & Hiley, 1988b).

In conclusion, this study shows that neuropeptide Y exerts its pressor effect in the pithed rat by increasing cardiac output and total peripheral resistance. The increased cardiac output is possibly through an increase in venous return, and the increased total peripheral resistance mainly due to mesenteric vasoconstriction. Neuropeptide Y, endothelin and the noradrenergic nervous system may interact in the control of blood pressure.

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References

- ALLEN, J.M., BIRCHAM, P.M.M., EDWARDS, A.V., TATEMOTO, K. & BLOOM, S.R. (1983). Neuropeptide Y (NPY) reduces myocardial perfusion and inhibits the force of contraction of the isolated perfused rabbit heart. *Reg. Pept.*, **6**, 247–253.
- 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.
- DALY, R.N. & HIEBLE, J.P. (1987). Neuropeptide Y modulates adrenergic neurotransmission by an endothelium dependent mechanism. *Eur. J. Pharmacol.*, **138**, 445–446.
- GARDINER, S.M., BENNETT, T. & COMPTON, A.M. (1988). Regional haemodynamic effects of neuropeptide Y, vasopressin and angiotensin II in conscious, unrestrained, Long Evans and Brattleboro rats. *J. Auton. Nerv. Syst.*, **24**, 15–27.
- GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1989). Regional haemodynamic effects of endothelin-1 in conscious, unrestrained, Wistar rats. *J. Cardiovasc. Pharmacol.*, **13**, (Suppl. 5), S202–S204.
- HAN, S.P., TRAPANI, A.J., FOK, K.F., WESTFALL, T.C. & KNUEPFER, M.M. (1989). Effects of endothelin on regional haemodynamics in conscious rats. *Eur. J. Pharmacol.*, **159**, 303–305.
- HARDAWAY, R.M. (1985). Treatment of shock from a clinical viewpoint. In *Circulatory Shock: Basic and Clinical Implications*. Research Topics in Physiology, Vol. 7, ed. Janssen, H.F. & Barnes, C.B., pp. 197–235. New York: Academic Press.
- 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.
- HOCK, C.E., PASSMORE, J.C., LEVIN, J.I. & NEIBERGER, R.E. (1982). Angiotensin II and α -adrenergic control of the intrarenal circulation in hemorrhage. *Circ. Shock*, **9**, 81–94.
- LUNDBERG, J.M., PERNOW, J., FRANCO-CERECEDA, A. & RUDEHILL, A. (1987). Effect of antihypertensive drugs on sympathetic vascular control in relation to neuropeptide Y. *J. Cardiovasc. Pharmacol.*, **10** (suppl. 12), S51–S68.
- LUNDBERG, J.M., SARIA, A., ÅNGGÅRD, A., HÖKFELT, T. & TERENIUS, L. (1984). Neuropeptide Y and noradrenaline interaction in peripheral cardiovascular control. *Clin. Exp. Theory and Practice*, **A6** (10, + 11), 1961–1972.
- LUNDBERG, J.M., TERENIUS, L., HÖKFELT, T. & GOLDSTEIN, M. (1983). High levels of neuropeptide Y in peripheral noradrenergic neurons in various mammals including man. *Neurosci. Lett.*, **42**, 167–172.
- MACLEAN, M.R. & HILEY, C.R. (1988a). Effect of artificial respiratory volume on the cardiovascular responses to an α_1 - and an α_2 -adrenoceptor agonist in the air-ventilated pithed rat. *Br. J. Pharmacol.*, **93**, 781–790.
- MACLEAN, M.R. & HILEY, C.R. (1988b). Effect of enalapril on changes in cardiac output and organ resistances induced by α_1 - and an α_2 -adrenoceptor agonists in the pithed rat. *Br. J. Pharmacol.*, **94**, 449–462.
- MACLEAN, M.R., RANDALL, M.D. & HILEY, C.R. (1989a). Effects of moderate hypoxia, hypercapnia and acidosis on haemodynamic changes induced by endothelin-1 in the pithed rat. *Br. J. Pharmacol.*, **98**, 1055–1065.
- MACLEAN, M.R., THOMSON, M. & HILEY, C.R. (1989b). Pressor effects of the α_2 -adrenoceptor agonist B-HT 933 in anaesthetised and haemorrhagic rats: comparison with the haemodynamic effects of amidephrine. *Br. J. Pharmacol.*, **97**, 419–432.
- MCDEVITT, D.G. & NIES, A.S. (1976). Simultaneous measurement of cardiac output and its distribution with microspheres in the rat. *Cardiovasc. Res.*, **10**, 494–498.
- MINSON, R.B., MCRTCHIE, R.J. & CHALMERS, J.P. (1987). Effects of neuropeptide Y on left ventricular function in the conscious rabbit. *Clin. Exp. Pharmacol. Physiol.*, **14**, 263–266.
- MORRIS, M.J., ELLIOTT, J.M., CAIN, M.D., KAPOOR, V., WEST, M.J. & CHALMERS, J.P. (1986). Plasma neuropeptide Y levels rise in patients undergoing exercise tests for the investigation of chest pain. *Clin. Exp. Pharmacol. Physiol.*, **13**, 437–440.
- 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.
- 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 anaesthetised rats. *Clin. Exp. Pharmacol. Physiol.*, **14**, 703–710.
- SNEDECOR, G.W. & COCHRAN, W.G. (1980). In *Statistical Methods*. 7th edition, pp. 215–233. Iowa: Iowa State University Press.
- TATEMOTO, K. (1982). Neuropeptide Y; the complete amino acid sequence of the brain peptide. *Proc. Natl. Acad. Sci., U.S.A.*, **79**, 5485–5489.
- THOMAS, G.R., WALDER, C.E., THIEMERMANN, C. & VANE, J.R. (1989). Effects of endothelin on cardiac output and regional blood flow in the pithed rat. *Br. J. Pharmacol.*, **96**, 166P.
- WALDER, C.E., THOMAS, G.R., THIEMERMANN, C. & VANE, J.R. (1989). The haemodynamic effects of endothelin-1 in the pithed rat. *J. Cardiovasc. Pharmacol.*, **13**, (Suppl. 5), S93–S97.
- WESTFALL, T.C., CARPENTER, 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.
- YANAGISAWA, M., KURIHARA, S., KIMURA, Y., TOMOBE, M., KOBAYASHI, Y., MITSUI, Y., YAZAKI, Y., GOTO, K. & MASAKI, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, **332**, 411–415.
- ZUKOWSKA-GROJEC, Z., MARKS, E.S. & HAASS, M. (1987). Neuropeptide Y is a potent vasoconstrictor and a cardiodepressant in rat. *Am. J. Physiol.*, **253**, H1234–H1239.

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