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The actions of the peptides, neuropeptide Y and peptide YY, on the vascular and capsular smooth muscle of the isolated, blood-perfused spleen of the dog

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1 The two peptides, neuropeptide Y (NPY) and peptide YY (PYY) were compared for potency on the vascular and extravascular smooth muscle of the isolated, blood-perfused spleen of the dog.

2 The only vascular response to both NPY and PYY was vasoconstriction; the maximum effect was to arrest splenic arterial blood flow completely.

3 On a molar basis both NPY and PYY were significantly more potent ($P < 0.01$) as splenic arterial vasoconstrictors than the transmitter noradrenaline (NA).

4 PYY was approximately 7 times more potent as a vasoconstrictor than NPY.

5 In contrast to their potency on the vascular smooth muscle, NPY and PYY were significantly ($P < 0.01$) less potent than NA in causing contraction of the splenic capsule. The two peptides were equipotent in eliciting this contraction.

Introduction

The two peptides, neuropeptide Y (NPY) and peptide YY (PYY), are composed of 36 amino acids, display considerable structural homology and are clearly related to pancreatic polypeptide (PP) (Tatemoto, 1982). However, whilst PP is almost devoid of any action on vascular smooth muscle, both NPY and PYY have been reported to possess significant vasoconstrictor potency (Lundberg & Tatemoto, 1982).

The biological distribution of the two peptides, NPY and PYY, is very different; whilst NPY appears to be present in most tissues receiving a sympathetic innervation (Uddman *et al.*, 1985), PYY is located only in paracrine cells of the colon and pancreas (Lundberg *et al.*, 1982). The histochemical localization of NPY-like immunoreactivity within the noradrenergic innervation of spleen tissue has been described (Lundberg *et al.*, 1985a; Fried *et al.*, 1986) and its release into the splenic venous drainage following sympathetic nerve stimulation has been demonstrated (Lundberg *et al.*, 1984; 1986).

The spleen of the dog possesses a rich sympathetic noradrenergic innervation to both the blood vessels and the capsule (Dahlstrom & Zetterstrom, 1965). In this species the capsule vigorously contracts to expel erythrocytes into the circulation to elevate the oxygen carrying capacity of the blood during strenuous exercise. The aim of the present experiments was to characterize the actions of both NPY and PYY on the vascular and extravascular smooth muscle of the dog's spleen and compare their potency at these sites with that of noradrenaline, the principal mediator of neurotransmission. Subsequently a physiological role for NPY and PYY is suggested, consistent with their biological activity and histochemical distribution.

Methods

The experiments were performed on 6 dogs (mean weight 27.1 ± 1.7 kg; range 20.5–32 kg) anaesthetized with an intravenous mixture of chloralose and urethane (50 and 500 mg kg^{-1} respectively) after induction with methohexitone sodium (6 mg kg^{-1}). The trachea was cannulated although respiration was always spontaneous. The right femoral vein was cannulated to administer additional anaesthetic or dextran if considered necessary. The right femoral artery was cannulated to provide blood samples for analysis of pH,

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P_{CO_2} and P_{O_2}; if appropriate $NaHCO_3$ (1 mmol min^{-1}) was infused i.v. to maintain normal blood pH. The left carotid artery was cannulated to provide a continuous registration of phasic systemic blood pressure from which a continuous recording of heart rate was derived electronically. Body temperature was maintained within normal limits, as indicated by a buccal thermometer, by either table heaters or overhead lamps.

The preparation for splenic perfusion has been described previously (Withrington *et al.*, 1980). Briefly, after a midline laparotomy, all connections between the spleen and other tissues such as the stomach, pancreas and omentum were tied and cut so that the spleen remained attached only by the splenic pedicle of the main splenic artery, splenic vein and postganglionic nerve trunk. Heparin was then administered (500 iu kg^{-1}, i.v.) and the splenic artery, vein and nerves cut between ligatures. The spleen was removed from the abdomen and the splenic artery and vein cannulated with the widest bore polythene tubing possible. The spleen was then placed in a perspex plethysmograph where the arterial perfusion was established at systemic pressure with blood derived from the left femoral artery. The splenic venous effluent drained into a circuit to the cannulated left femoral vein and so returned to the animal. Incorporated into the splenic arterial circuit was a pressure transducer (Statham P23Gb) and a cannulating electromagnetic flow probe (Cardiovascular Instruments) to measure splenic arterial phasic perfusion pressure and splenic arterial phasic blood flow respectively. Both these signals were heavily damped to provide a continuous recording of splenic arterial mean perfusion pressure (SAPP) and splenic arterial mean blood flow (SABF) and from these values calculations of changes in splenic arterial vascular resistance (SAVR) could be made. A diversion was available in the arterial circuit for intermittent establishment of the zero position for the arterial flowprobe without interruption of the blood supply to the spleen. The flow probe was always calibrated with whole blood at the end of each experiment. A 'T' piece was also incorporated to allow close-arterial injections to be made.

The plethysmograph was filled with liquid paraffin and sealed with a perspex lid. A wide bore polythene tubing connected the contents of the plethysmograph to a perspex cylindrical reservoir suspended from an isometric sensor. When the spleen changed size the amount of liquid paraffin in the reservoir either increased or decreased. The continuous registration of the reservoir weight therefore provides a direct assessment of changes in spleen volume. The system was calibrated by adding or removing known volumes of liquid paraffin to the reservoir. The level of the liquid paraffin in the reservoir determines the pressure within the plethysmograph and therefore the external pressure on the spleen. The level of the liquid paraffin in the reservoir was therefore maintained at the same level as the splenic pedicle and the capacity of the reservoir was such that the level did not alter by more than 5 mm during the experiment.

At the end of each experiment the splenic artery and vein were clamped; the spleen removed from the

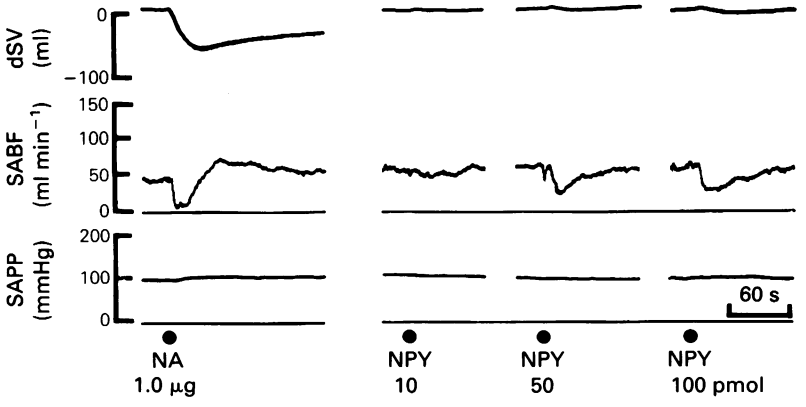

Figure 1 Isolated, blood perfused spleen (366 g) of the dog. Records are from the top; dSV, reduction in spleen volume; SABF, splenic arterial mean blood flow; SAPP, splenic arterial mean perfusion pressure. The four panels illustrate the changes in response to intra-arterial injections of noradrenaline (NA, 1.0 μg) and 3 doses of neuropeptide Y (NPY, 10, 50 and 100 pmol).

plethysmograph and weighed after removal of blood from the organ. Splenic arterial blood flow and resistance could then be expressed in terms of unit weight of spleen tissue.

Drugs used and vehicles

Noradrenaline, NPY and PYY were injected directly into the splenic arterial line through a 'T' piece. Test substances were washed in with saline (0.9% w/v NaCl solution) to give a total injectate of 2.0 ml. NPY and PYY were purchased from Bachem and made up in sterile saline which contained human serum albumin (10 mg ml^{-1}; Elstree) and Polypep (2.5 mg ml^{-1}; low viscosity; Sigma). Close arterial injection of this vehicle produced no changes in splenic arterial vascular resistance. The human serum albumin and Polypep were used to reduce non-specific binding of NPY and PYY onto plastic surfaces. Noradrenaline acid tartrate (Levophed; Winthrop) was diluted before injection in normal saline.

Statistics

Results are presented as means ± standard errors of mean (s.e.mean). Tests for significance refer to Student's *t* tests.

Results

Control values

The mean spleen weight was 355 ± 55 g representing 1.3 ± 0.18% of the total body weight. The initial mean splenic arterial mean blood flow was 114 ± 21 ml min^{-1} or 35.7 ± 12 ml min^{-1} 100 g^{-1}. Since the mean splenic arterial perfusion pressure was 119 ± 9.5 mmHg the mean calculated splenic arterial vascular resistance was 1.29 ± 0.3 mmHg ml^{-1} min or 5.00 ± 1.34 mmHg ml^{-1} min 100 g spleen weight. These values are slightly different from those previously reported from this laboratory and may reflect the use of larger animals and the variable extent to which both the arterial supply and venous drainage of the spleen are interrupted by the surgical procedures necessary for complete isolation.

Splenic vascular and capsular responses to close-arterial injections

Noradrenaline In all the preparations graded bolus injections of NA were made into the splenic arterial perfusion line over the dose range of 0.01–10 μg. The characteristic response (Figures 1 and 2) was a biphasic change in splenic arterial blood flow consisting of an initial rapid reduction in flow succeeded by a more prolonged secondary increase in flow. Since the mean perfusion pressure remained constant these changes represent splenic arterial vasoconstriction and vasodilatation respectively. These vascular changes are related to dose in a complex manner since they represent the overlapping responses to differential α- and β_2-adrenoceptor activation. Concomitant with these vascular effects was a rapid contraction of the splenic capsular smooth muscle resulting in a reduction in spleen volume. The duration of this response usually outlasted the biphasic vascular changes in splenic arterial flow.

These excitatory effects, splenic arterial vasoconstriction and capsular contraction are dose-related (Figure 3). The threshold dose was the same for both responses (less than 1.0 nmol NA). The maximum

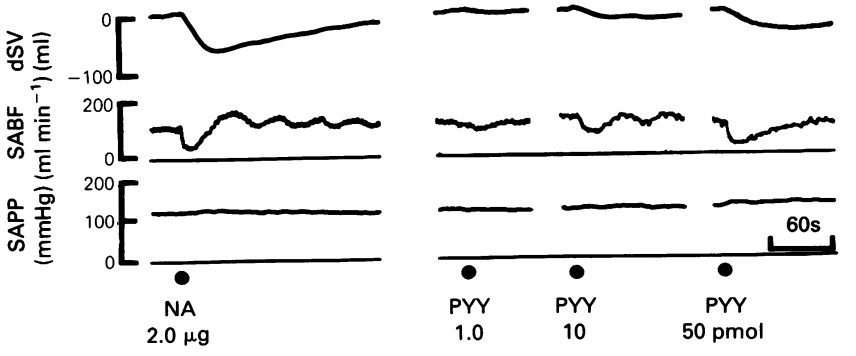

Figure 2 Isolated, blood perfused spleen (486 g) of the dog. The records are the same as in Figure 1; dSV (reduction in spleen volume), SABF (splenic arterial mean blood flow) SAPP (splenic arterial mean perfusion pressure). The four panels illustrate the responses of the splenic capsular and vascular smooth muscle to the close-arterial injections of noradrenaline (NA, 2.0 μg) and peptide YY (PYY, 1.0, 10 and 50 pmol).

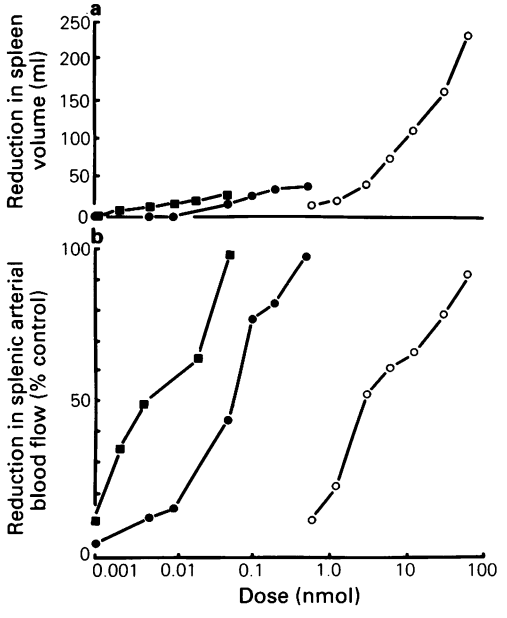

Figure 3 Isolated, blood perfused spleen (214 g) of dog. The dose-response curves relate the reductions in spleen volume (a) and splenic arterial blood flow (b) to the molar dose of peptide YY (PYY, ■), neuropeptide Y (NPY, ●) and noradrenaline (NA, O) administered as close-arterial injections. All the points were obtained in the same experiment under comparable control conditions of perfusion pressure (121 ± 2.1 mmHg) arterial inflow (162 ± 5.5 ml min^{-1}) and splenic vascular resistance (0.76 ± 0.025 mmHg ml^{-1} min).

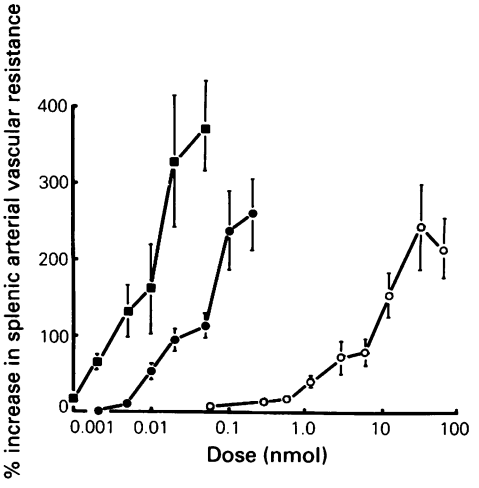

Figure 4 The relationship obtained in 6 separate spleen perfusions, between graded molar doses of intra-arterial peptide YY (PYY, ■), neuropeptide Y (NPY, ●) and noradrenaline (NA, O) and the increase in splenic arterial vascular resistance (splenic vasoconstriction) as a percentage of the control. Not all the doses were administered in each experiment and so the points and bars represent the means and s.e. of between 3 and 6 observations.

splenic responses were achieved at either 50–100 nmol when complete arrest of the splenic inflow occurred and there was a substantial reduction in speen volume (mean maximum reduction = 129 ± 29 ml). The ED_{50} for the vascular effect (vasoconstriction) was 12.5 ± 4.7 nmol for NA.

Neuropeptide Y In 6 preparations NPY was injected into the splenic arterial circuit in graded doses over the range 1.0–500 pmol to establish the majority of the dose-response curve. The only vascular response observed was a reduction in splenic arterial blood flow which, without any accompanying changes in perfusion pressure, is indicative of splenic arterial vasoconstriction. This splenic vasoconstrictor effect was graded with dose (Figure 1).

The time course of the vasoconstrictor response to NPY was different from that to NA. The onset of the response was slightly slower and the recovery from the maximum rather more prolonged. In most preparations the threshold dose was 5–10 pmol (Figures 3 and 4) and the maximum vascular effect, complete cessation of splenic arterial inflow, was obtained at doses of 200–500 pmol (Figure 3). In all experiments the dose of NPY required to reduce splenic arterial blood flow to 50% of the control value was assessed from the constructed dose-response in that experiment. The mean value was 31.8 ± 7.1 pmol; a value very significantly less ($P < 0.001$) than the calculated mean ED_{50} for NA.

In contrast to injected NA, there were either no or very small accompanying changes in spleen volume to close-arterial injections of NPY (Figures 1 and 3). In most experiments the splenic capsular response to high doses of NPY was less than 20% of that to NA when the splenic vascular response was the same and almost maximal. The mean maximum reduction in spleen volume to close-arterial injections of NPY was 23.7 + 5.0 ml; which was significantly less ($P < 0.01$) than that achieved with NA.

Peptide YY In 3 preparations PYY was injected into the splenic arterial circuit in graded bolus doses over the range of 1.0–100 pmol to construct 4 complete dose-response curves. The only vascular response to any injection of PYY was a reduction in splenic arterial blood flow which at constant perfusion pressure indicates a rise in splenic arterial vascular resistance and therefore splenic arterial vasoconstriction (Figure 2). This vasoconstrictor response to PYY was

graded with dose and in most experiments had a time course slightly longer than that of equipotent doses of noradrenaline. The threshold for the splenic vasoconstrictor response was, in some preparations, below 1.0 pmol whilst the maximum vascular effect, i.e. complete arrest of splenic arterial blood flow, was achieved with 100 pmol (Figure 3). In those preparations where dose-response curves to both PYY and NPY were constructed, the effective range (i.e. threshold to maximum) of intra-arterial doses was always less for PYY than for NPY. The mean dose of PYY necessary to reduce the splenic arterial flow to 50% of control value was assessed from the individual dose-response curves. The value of 4.45 ± 0.51 pmol was significantly lower than the value for NPY (31.8 ± 7.1 pmol; $P < 0.01$).

In contrast to the responses to NA, PYY (like NPY) produced only small reductions in spleen volume (Figure 2), even when the concomitant splenic arterial vascular response was almost maximal (Figure 3) indicative of minimal actions of the peptide on the splenic capsular smooth muscle. The mean maximum reduction in spleen volume to intra-arterial PYY was 16.8 ± 8.2 ml, a value not significantly different from the maximum to NPY ($P > 0.5$) but significantly less than the maximum reduction in spleen volume caused by NA ($P < 0.01$).

Discussion

It is widely accepted that NA is the principal catecholamine neurotransmitter at the sympathetic terminals innervating the smooth muscle of the vasculature and capsule of the spleen. The postsynaptic receptors mediating the excitatory responses of splenic arterial vasoconstriction and capsular contraction belong to the α-adrenoceptor class on the basis of the action of α-adrenoceptor antagonist drugs (Davies *et al.*, 1969), whilst the secondary splenic arterial vasodilator response is due to β_2-adrenoceptor activation since the vasodilator action of isoprenaline is antagonized by the selective β_2-antagonist ICI 118, 551 (Withrington, unpublished observations).

A number of biologically active peptides are co-stored with the principal transmitter and these include NPY (Fried *et al.*, 1986). This peptide is one member of a family of structurally related hexatriacontapeptides comprising NPY, PYY and the pancreatic polypeptides (PPs) (Tatemoto, 1982). Co-release of NPY with noradrenaline during sympathetic nerve stimulation has been demonstrated by measuring the concentrations of these two substancs in the venous effluents from feline (Lundberg *et al.*, 1984), porcine (Lundberg *et al.*, 1986) and canine (Corder, Lowry & Withrington, unpublished) spleen. The neuronally released NPY in the spleen may have an important role in vascular sympathetic neurotransmission either through a direct vasoconstrictor action (Lundberg *et al.*, 1985a), by enhancing the noradrenergic response (Wahlestedt *et al.*, 1985), or by regulating presynaptically the release of noradrenaline (Lundberg *et al.*, 1985b).

Bolus administration of NPY has been shown to elicit a systemic pressor effect which is resistant to adrenoceptor blockade (Lundberg & Tatemoto, 1982; Dahlof *et al.*, 1985; Corder *et al.*, 1986). This effect is due almost entirely to an increase in peripheral vascular resistance (Corder *et al.*, 1986). However, the importance of individual tissue sensitivity to NPY in producing changes in regional vascular resistance has not been adequately investigated. In addition, the *in vitro* action of NPY has been shown to vary to some extent, depending on the source of the vascular smooth muscle. Thus, intrinsic vasoconstrictor activity of NPY has been observed on feline cerebral arteries (Edvinsson *et al.*, 1983) and guinea-pig iliac vein (Wahlestedt *et al.*, 1986) whilst on many arterial preparations potentiation of the noradrenergic responses appeared to be the main effect (Edvinsson *et al.*, 1984; Wahlestedt *et al.*, 1986).

In the experiments described here, close arterial injections of NPY into the spleen revealed a high molar potency for the peptide in exciting the splenic arterial vascular smooth muscle, since it evoked splenic arterial vasoconstriction with a prolonged time-course. In contrast the peptide had little effect on the capsular smooth muscle. This differential action of NPY at two smooth muscle sites within the same structure is not unique and reflects the predominant activity of other peptides such as vasopressin and angiotensin in this organ (Davies & Withrington, 1975). It may also reflect the differential histochemical localisation of NPY-like immunoreactivity within the spleen: high density in the vasculature but largely absent from the capsular innervation (Lundberg *et al.*, 1985; Fried *et al.*, 1986).

Although NPY is the endogenous spleen peptide, PYY is some seven times more potent in producing splenic vascular vasoconstriction (Figure 4). Similar differences in potencies for these two peptides have been reported for the systemic pressor action in cats (Lundberg & Tatemoto, 1982) and the presynaptic inhibitory effect on rat vas deferens (Chang *et al.*, 1985); but in a study of radiolabelled NPY binding to rat brain membrane preparations, PYY and NPY had almost identical potencies (IC_{50}) for the inhibition of binding (Chang *et al.* 1985). If the direct vasoconstrictor effects of NPY and PYY are elicited through the same receptor, the difference in potency may be attributed to either PYY having greater intrinsic activity or alternatively PYY may be less rapidly degraded by proteolytic enzymes than NPY. That is, a specific vascular enzyme for the metabolism of NPY

may be present in the spleen, but the small structural differences in PYY may afford a degree of resistance to enzyme degradation. The absolute potency ratio of NPY, PYY and NA as vasoconstrictors will, in the intact animal, depend on a wide variety of other factors including uptake mechanisms and the presence of other receptor agonists. Differential interactions between NPY, PYY and other endogenous peptides need to be evaluated to provide a quantitative estimate of their relative potency at any particular site of action.

In conclusion, the vasoconstrictor activity of PYY, NPY and NA have been compared in the splenic vasculature. This has shown that PYY and NPY are approximately 2,800 and 400 times more potent respectively on a molar basis than NA as splenic arterial vasoconstrictor agents. However, unlike NA, PYY and NPY are almost without effect on capsular smooth muscle.

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References

CHANG, R.S.L., LOTTI, V.J., CHEN, T., CERINO, D.J. & KLING, P.J. (1985). Neuropeptide Y (NPY) binding sites in rat brain labelled with 125I-Bolton-Hunter NPY: comparative potencies of various polypeptides on brain NPY binding and biological responses in the rat vas deferens. *Life Sci.*, **37**, 2111–2122.

CORDER, R., LOWRY, P.J., WILKINSON, S.J. & RAMAGE, A.G. (1986). Comparison of the haemodynamic actions of neuropeptide Y, angiotensin II and noradrenaline in anaesthetised cats. *Eur. J. Pharmac.*, **121**, 25–30.

DAHLOF, C., DAHLOF, 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. Pharmac.*, **109**, 289–292.

DAHLSTROM, A.B. & ZETTERSTROM, B.E.M. (1965). Noradrenaline stores in nerve terminals of the spleen; changes during haemorrhagic shock. *Science*, **147**, 1583–1585.

DAVIES, B.N., ROBINSON, B.H. & WITHRINGTON, P.G. (1969). Effects of graded doses of phenoxybenzamine on the vascular and capsular responses of the isolated blood perfused spleen of the dog to sympathetic nerve stimulation and catecholamines. *Archs int. Pharmacodyn.*, **180**, 143–154.

DAVIES, B.N. & WITHRINGTON, P.G. (1975). The responses of the isolated blood perfused spleen of the dog to angiotensin, oxytocin and vasopressin. *Br. J. Pharmac.*, **54**, 205–212.

EDVINSSON, L., EMSON, P.C., McCULLOCH, J., TATEMOTO, K. & UDDMAN, R. (1983) Neuropeptide Y: cerebrovascular innervation and vasomotor effects in the cat. *Neurosci. Letts.*, **43**, 79–85.

EDVINSSON, L., EKBLAD, E., HAKANSON, R. & WAHLESTEDT, C. (1984). Neuropeptide Y potentiates the effect of various vasoconstrictor agents on rabbit blood vessels. *Br. J. Pharmac.*, **83**, 519–525.

FRIED, G., TERENIUS, L., BRODIN, E., EFENDIC, S., DOCKRAY, G., FAHRENKRUG, J., GOLDSTEIN, M. & HOKFELT, T. (1986). Neuropeptide Y, enkephalin and noradrenaline coexist in sympathetic neurons innervating the bovine spleen. *Cell Tissue Res.*, **243**, 495–508.

LUNDBERG, J.M. & TATEMOTO, K. (1982). Pancreatic polypeptide family (APP, BP, NPY and PYY) in relation to sympathetic vasoconstriction resistant to alpha-adrenoceptor blockade. *Acta physiol. scand.*, **116**, 393–402.

LUNDBERG, J.M., TATEMOTO, K., TERENIUS, L., HELLSTROM, P.M., MUTT, V., HOKFELT, T. & HAMBERGER, B. (1982). Localization of peptide YY (PYY) in gastrointestinal endocrine cells and effects on intestinal blood flow and motility. *Proc. natn. Acad. Sci., U.S.A.*, **79**, 4471–4475.

LUNDGERG, J.M., ANGARD, A., THEODORSSON-NORHEIM, E. & PENROW, J. (1984). Guanethidine-sensitive release of neuropeptide Y-like immunoractivity in the cat spleen by sympathetic nerve stimulation. *Neurosci. Letts.*, **52**, 175–180.

LUNDBERG, J.M., ANGGARD, A., PERNOW, J. & HOKFELT, T. (1985a). Neuropeptide Y-, substance P- and VIP-immunoreactive nerves in cat spleen in relation to autonomic vascular and volume control. *Cell Tissue Res.*, **239**, 9–18.

LUNDBERG, J.M., PERNOW, J., TATEMOTO, K. & DAHLOF, C. (1985b). Pre- and postjunctional effects of NPY on sympathetic control of rat femoral artery. *Acta physiol. scand.*, **123**, 511–513.

LUNDBERG, J.M., RUDEHILL, A., SOLLEVI, A., THEODORSSON-NORHEIM, E. & HAMBERGER, B. (1986). Frequency- and reserpine-dependent chemical coding of sympathetic transmission: differential release of noradrenaline and Neuropeptide Y from pig spleen. *Neurosci, Letts.*, **63**, 96–100.

TATEMOTO, K. (1982). Neuropeptide Y: complete amino acid sequence of the brain peptide. *Proc. natn. Acad. Sci. U.S.A.*, **79**, 5485–5489.

UDDMAN, R., EKBLAD, E., EDVINSSON, L., HAKANSON, R. & SUNDLER, F. (1985). Neuropeptide Y-like immunoreactivity in perivascular nerve fibres of the guinea-pig. *Regulatory Peptides*, **10**, 243–257.

WAHLESTEDT, C., EDVINSSON, L., EKBLAD, E. & HAKANSON, R. (1985). Neuropeptide Y potentiates noradrenaline-evoked vasoconstriction: mode of action. *J. Pharmac. exp. Ther.*, **234**, 735–741.

WAHLESTEDT, C., YANAIHARA, N. & HAKANSON, R. (1986). Evidence for different pre- and post-junctional receptors for neuropeptide Y and related peptides. *Regulatory Peptides.*, **13**, 307–318.

WITHRINGTON, P.G., RICHARDSON, P.D.I. & DAVIES, B.N. (1980). Erythrocyte uptake by the dog spleen during splenic venous elevation. *Pflugers Arch.*, **388**, 37–43.

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