Br. J. Pharmacol. (1990), **99**, 389–395

Enhancement by neuropeptide Y (NPY) of the dihydropyridine-sensitive component of the response to α_1-adrenoceptor stimulation in rat isolated mesenteric arterioles

Ramaroson Andriantsitohaina & [1]Jean Claude Stoclet

Laboratoire de Pharmacologie Cellulaire et Moléculaire (URA CNRS D0600), Université Louis Pasteur de Strasbourg, Faculté de Pharmacie, B.P. 24, F - 67401 Illkirch Cedex, France

1 The mechanism by which neuropeptide Y (NPY) potentiates the vasoconstriction induced by α_1-adrenoceptor agonists was investigated in 3rd generation mesenteric arterioles of the rat.

2 At a maximally active concentration, nitrendipine (10^{-6} M) displaced to the right the concentration-response curves to noradrenaline (pD_2 decreased from 6.2 ± 0.06 to 5.7 ± 0.03) and phenylephrine (pD_2 decreased from 5.6 ± 0.03 to 5.3 ± 0.03). Diltiazem (10^{-5} M) also shifted to the right the concentration-response curve to phenylephrine (pD_2 decreased from 6.0 ± 0.06 to 5.5 ± 0.04). In addition, the maximal response to phenylephrine was significantly decreased in the presence of either nitrendipine or diltiazem.

3 In the absence of a calcium channel blocking agent, NPY (100 nM) produced a leftward shift of the concentration-response curves to noradrenaline (pD_2 increased from 6.2 ± 0.06 to 6.5 ± 0.05) and phenylephrine (pD_2 increased from 5.6 ± 0.03 to 6.0 ± 0.06 and from 6.0 ± 0.06 to 6.3 ± 0.11). In the presence of either nitrendipine (10^{-6} M) or diltiazem (10^{-5} M), NPY (100 nM) did not alter the concentration-response curves to either noradrenaline or phenylephrine.

4 NPY was added to arterioles brought to the same level of tension (40% of the maximal contraction) either by phenylephrine alone (1.5×10^{-6} M) or by a higher concentration of phenylephrine (3×10^{-6} M) followed by the addition of prazosin (1.3×10^{-9} M; a concentration at which it partially blocks α_1-adrenoceptors). In these conditions, the response to phenylephrine was completely abolished by nitrendipine (10^{-6} M) or by diltiazem (10^{-5} M). Furthermore, NPY (10^{-10} to 10^{-7} M) increased the arteriolar tension up to the maximal contractile capacity of the vessels with pD_2 values of 8.6 ± 0.02 and 8.7 ± 0.01, in the absence and presence of prazosin, respectively.

5 Prazosin was replaced in the above protocol by other vasodilator agents acting through different mechanisms. Whether in the presence of 2×10^{-7} M forskolin, 6×10^{-7} M sodium nitroprusside (which stimulate adenylate cyclase or guanylate cyclase, respectively) or 2×10^{-7} M diltiazem (a concentration at which calcium entry is partially blocked), NPY enhanced phenylephrine-induced contraction to the maximum level with an identical potency (pD_2 values of the peptide ranged from 8.3 to 8.7).

6 The results show that, in rat mesenteric arterioles, NPY potentiates only the calcium entry blocker-sensitive component of contraction induced by stimulation of α_1-adrenoceptors. In addition, they provide evidence that the peptide counteracts with an equal potency the inhibitory effect of partial block of α_1-adrenoceptors and of relaxing agents acting through different mechanisms. It is suggested that NPY enhances calcium entry induced by stimulation of α_1-adrenoceptors in this tissue.

Introduction

Neuropeptide Y (NPY) is a potent vasoconstrictor peptide which is co-stored with catecholamines in sympathetic nerve endings. It is also co-released with noradrenaline upon sympathetic nerve stimulation (see reviews, O'Donohue *et al.*, 1985; Allen & Bloom, 1986; Gray & Morley, 1986; Sundler *et al.*, 1986; Edvinsson *et al.*, 1987; Lundberg *et al.*, 1987; Potter, 1988). Systemic administration of NPY to conscious rats increase blood pressure and this is associated with an increase in total peripheral resistance (Dählof *et al.*, 1985; Zukowska-Grojec *et al.*, 1986; Pegram & Hunter, 1988).

The effects of NPY on the various vascular beds appear to be diverse. It possesses a direct vasoconstrictor action in some but not all isolated vessels and it reduces prejunctional release of noradrenaline. However, at the postjunctional level, the main property of this peptide is its ability to potentiate responses caused either by nerve stimulation or by a variety of vasoconstrictor agents, including noradrenaline (Lundberg & Tatemoto, 1982; Lundberg *et al.*, 1982; Edvinsson *et al.*, 1984a,b; Ekblad *et al.*, 1984; Edvinsson, 1985; Glover, 1985; Wahlestedt *et al.*, 1985; 1986; Zukowska *et al.*, 1987; Han & Abel, 1987; Neild, 1987; 1988; Juan *et al.*, 1988).

In a previous study on rat isolated mesenteric resistance arterioles, NPY enhanced contraction elicited by a number of agonists or by KCl-depolarization, at a concentration which produced no effect by itself. It was suggested that these effects could be accounted for by partial depolarization and subsequent calcium influx through voltage-dependent calcium channels (Andriantsitohaina & Stoclet, 1988a). Abel & Han (1989) reached similar conclusions on rabbit cerebral arteries. However, two other mechanisms have been proposed to explain the potentiating effect of NPY on vasoconstriction induced by noradrenaline: inhibition of adenylate cyclase activity (Fredholm *et al.*, 1985; Häggblad & Fredholm, 1987; Lundberg *et al.*, 1988) and enhanced accumulation of inositol trisphosphate (Häggblad & Fredholm, 1987).

In the present work, the action of NPY was further investigated in rat mesenteric arterioles. Experiments have been carried out in order to examine the ability of the peptide to amplify the dihydropyridine-sensitive and -insensitive components of the responses to two α_1-adrenoceptor agonists, noradrenaline and phenylephrine; these two components of the response are produced through different pathways and

[1] Author for correspondence.

might even be due to stimulation of different α_1-adrenoceptor subtypes (Han *et al.*, 1987). In addition, the capacity of the peptide to enhance contraction in the presence of various vasodilator agents acting through different mechanisms was also studied. The results show that the peptide did not potentiate the dihydropyridine-insensitive component of the agonist-induced contractions, but was able to potentiate the component which is sensitive to calcium entry blockers. For this latter component, NPY was also able to counteract the inhibitory effect of all vasodilator agents tested. It is suggested that NPY enhances the efficiency of coupling between receptor occupancy and calcium entry through a mechanism sensitive to dihydropyridines and diltiazem.

Part of this work was presented at the December 1988 meeting of the British Pharmacological Society (Andriantsitohaina & Stoclet, 1988b).

Methods

Arterial preparation

In all experiments, third generation branches of the superior mesenteric artery were dissected from 12–14 week old female Wistar rats bred in our institute. Rats were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, i.p.). Arterial segments (length = 2 mm, internal diameter = 100 μm) were prepared and mounted in a myograph (Mulvany & Halpern, 1977; Julou & Freslon, 1986). Briefly, two tungsten wires (30 μm diameter) were inserted through the lumen of the vessel. Mechanical activity was recorded isometrically by a force transducer (DSG BE4, Kistler–Morse), connected to one of the two tungsten wires, the other being attached to a support carried by a micromanipulator (MR 50, Micro–Contrôle).

After being set up, vessels were equilibrated for 1 h in a physiological salt solution (PSS) (composition in mM: NaCl 119, KCl 4.7, KH_2PO_4 0.4, $NaHCO_3$ 14.9, $MgSO_4$ 1.17, $CaCl_2$ 2.5, glucose 5.5) kept at 37°C and continuously gassed with a 95% O_2, 5% CO_2 mixture (pH 7.4). The resting tension of the preparation was adjusted to about 200 mg. Following equilibration, the contractile capacity was tested by exposing the arterial segment to 10^{-5} M noradrenaline, a concentration which elicited maximum contractions (Mulvany *et al.*, 1982). The presence of functional endothelium was checked in all preparations by the ability of acetylcholine (10^{-6} M) to induce more than 50% relaxation of vessels precontracted with noradrenaline (10^{-5} M).

Effects of NPY on noradrenaline- and phenylephrine-induced contractile responses in the absence and in the presence of a calcium entry blocker

Sixty min after the contractile capacity of the vessels had been tested with noradrenaline, concentration-response curves were constructed to either noradrenaline or phenylephrine. After a washout period of 45 min, NPY (100 nM) was applied 5 min before the cumulative addition of the agonist was repeated. When a calcium channel blocker (10^{-6} M nitrendipine or 10^{-5} M diltiazem) was used, it was added 20 min before addition of the agonist. During experiments with noradrenaline, cocaine (3×10^{-6} M) was present in the bath to inhibit neuronal uptake. In control experiments in the absence of NPY, two concentration-response curves obtained by successive cumulative addition of the agonists were not different from each other.

Effect of NPY on vessels precontracted with phenylephrine

Sixty min after the contractile capacity of the vessels had been tested with noradrenaline, concentration-response curves to NPY (10^{-10} to 10^{-7} M) were constructed non-cumulatively by the addition of a single concentration of the neuropeptide to an arteriole precontracted with 1.5×10^{-6} M phenylephrine. When the contraction to NPY had reached equilibrium, both the phenylephrine and the neuropeptide were washed out and the preparation left 30 min before the addition of the same concentration of phenylephrine and a different concentration of the neuropeptide.

Effects of prazosin, forskolin, sodium nitroprusside and diltiazem on vessels precontracted with phenylephrine

Sixty min after the test addition of noradrenaline, the arterioles were precontracted with 3×10^{-6} M phenylephrine. When the response was stable, the arterioles were relaxed by addition of either prazosin(10^{-10} to 10^{-7} M), forskolin (10^{-8} to 10^{-5} M) sodium nitroprusside (10^{-8} to 10^{-5} M) or diltiazem (10^{-8} to 10^{-4} M). Concentration-response curves to each agent were constructed non-cumulatively by the addition of a single concentration of the chosen vasodilator to an arteriole precontracted with 3×10^{-6} M phenylephrine (except nitrendipine). When the relaxation had reached equilibrium, both the phenylephrine and the vasodilator were washed out and the preparation left 30 min before the addition of the same concentration of phenylephrine and a different concentration of the vasorelaxant. In control experiments, it was found that the responses induced by 3×10^{-6} M phenylephrine were consistent over the period of the experiment and did not differ significantly between preparations.

Effects of NPY on relaxation induced by prazosin, forskolin, sodium nitroprusside and diltiazem on vessels precontracted with phenylephrine

Sixty min after the contractile capacity of the vessels had been tested by exposure to 10^{-5} M noradrenaline, responses to NPY were elicited on arterioles which had first been precontracted with 3×10^{-6} M phenylephrine and then relaxed by different vasodilator agents (prazosin, forskolin, sodium nitroprusside or diltiazem), at the concentrations at which they produced half-maximal relaxation before addition of NPY. Concentration-response curves to NPY (10^{-10} to 10^{-7} M) were then constructed non-cumulatively by the addition of a single concentration of NPY to an arteriole precontracted with 3×10^{-6} M phenylephrine and relaxed by the chosen agent. When the response to NPY had reached equilibrium, phenylephrine, the vasodilator and NPY were washed out and the preparation left 30 min before the addition of the same concentrations of phenylephrine and the vasorelaxant but a different concentration of NPY. Control experiments were run in parallel; the relaxations induced by the vasodilator agents were not significantly different from each other and were consistent over the period of the experiments in the absence of NPY.

Expression of results and statistical analysis

Sensitivities to the agonists (noradrenaline, phenylephrine and NPY) and to the relaxant agents (forskolin, sodium nitroprusside, prazosin, diltiazem and nitrendipine) were expressed as pD_2 values, where $pD_2 = -\log EC_{50}$, EC_{50} being the concentration of agonist or relaxant agent required to give half-maximal response of the vessels. EC_{50} values were obtained by logit/log regression analysis.

All results are expressed as mean ± s.e.mean of n experiments. The differences between the pD_2 values, obtained in the same artery rings, were tested for significance by use of Student's t test for paired observations. One-way analysis of variance was used to compare the effect of different treatments studied in separate experiments.

Drugs

Noradrenaline bitartrate (Sigma) was dissolved in deionized water, containing 34 mM HCl and 7.9 mM Na_2SO_3, to give a

stock solution of 10^{-2} M. Acetylcholine hydrochloride (Sigma) was prepared as a 10^{-2} M stock solution by dissolving it in deionized water containing NaH_2PO_4 (0.1 M). Phenylephrine hydrochloride (Sigma), diltiazem hydrochloride (L.E.R.S.Synthelabo) and cocaine hydrochloride (Cooperation Pharmaceutique Française) were prepared daily as 10 mM solutions in deionized water. Forskolin (Sigma) and sodium nitroprusside (Merck) were diluted in absolute ethanol as 10 mM solutions. Porcine NPY (Sigma) was dissolved in deionized water containing 9 g l^{-1} NaCl and 1 g l^{-1} bovine serum albumin (Sigma) to give a stock solution of 10^{-4} M, this was subsequently stored frozen until use. Nitrendipine was dissolved in absolute ethanol to give a 1 mM stock solution which was kept frozen until use. Nitrendipine was kept in the dark and experiments with this drug were performed in light-proof apparatus to minimize light-induced degradation.

Results

The influence of nitrendipine and diltiazem on the responses to cumulative additions of noradrenaline and phenylephrine

As illustrated in Figure 1, the responses of the arterioles to cumulative additions of noradrenaline and phenylephrine were altered in the presence of the calcium entry blockers nitrendipine, at a maximally active concentration (10^{-6} M), and diltiazem (10^{-5} M). In the absence of a calcium entry blocker, contractions reached a sustained plateau after each addition of agonist, whereas in their presence, contractions reached a maximum within the first 30 s and then decreased progressively with time. In these conditions, nitrendipine (10^{-6} M) shifted the concentration-response curves for both noradrenaline and phenylephrine to the right (Figure 2a and

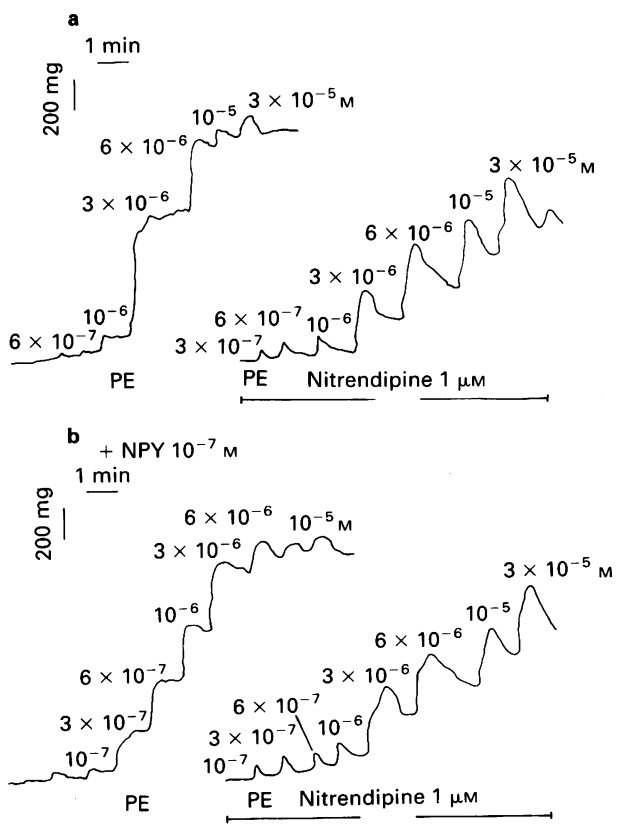

Figure 1 The influence of nitrendipine (10^{-6} M) on the contractile response of rat isolated mesenteric arterioles to cumulative addition of phenylephrine (PE) in the absence (a) and in the presence (b) of neuropeptide Y (NPY, 10^{-7} M). Similar traces were obtained in the presence of diltiazem (10^{-5} M).

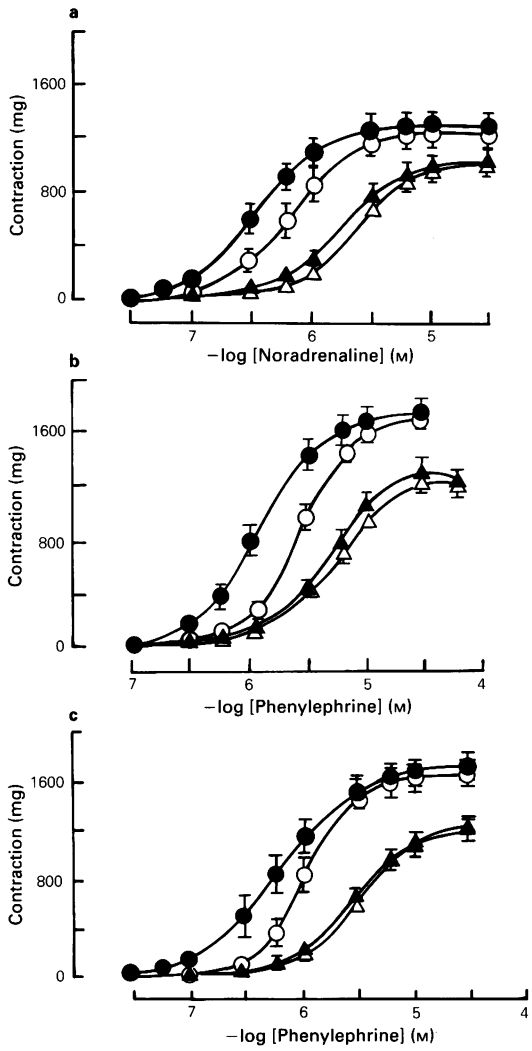

Figure 2 Effect of neuropeptide Y (NPY, 10^{-7} M) on contraction elicited by noradrenaline in the absence and presence of nitrendipine (10^{-6} M; a, $n = 10$), and by phenylephrine in the absence and presence of either nitrendipine (10^{-6} M; b, $n = 5$) or diltiazem (10^{-5} M; c, $n = 6$). Control curves (○), in the presence of NPY (●), in the presence of the calcium antagonist (△) and in the presence of NPY plus the calcium antagonist (▲). The values are the mean with the error bars representing s.e.mean.

b), diltiazem (10^{-5} M) also shifted the concentration-response curves for phenylephrine to the right (Figure 2c). The pD_2 values were decreased in the presence of nitrendipine from 6.2 ± 0.06 to 5.7 ± 0.03 (noradrenaline, $P < 0.001$) and from 5.6 ± 0.03 to 5.3 ± 0.03 M (phenylephrine, $P < 0.001$). The pD_2 values of phenylephrine were also decreased in the presence of diltiazem from 6.0 ± 0.06 to 5.5 ± 0.04 ($P < 0.001$). Nitrendipine (10^{-6} M) did not significantly affect the maximal response to noradrenaline but it decreased the maximal responses induced by phenylephrine ($P < 0.01$). Diltiazem (10^{-5} M) significantly decreased ($P < 0.01$) the maximal response induced by phenylephrine to the same extent as nitrendipine (10^{-6} M).

In the absence of a calcium entry blocker, NPY (10^{-7} M; a concentration at which it had no contractile effect by itself), produced significant leftward shifts of the noradrenaline and phenylephrine concentration-response curves, without changing the maximal responses (Figure 2). The pD_2 values were significantly enhanced by NPY from 6.2 ± 0.06 to 6.5 ± 0.05 ($P < 0.01$) for noradrenaline (Figure 2a), from 5.6 ± 0.03 to 6.0 ± 0.06 ($P < 0.001$) for phenylephrine (Figure 2b) and from 6.0 ± 0.06 to 6.3 ± 0.11 ($P < 0.05$) also for phenylephrine (Figure 2c). In contrast, in the presence of nitrendipine or diltiazem NPY did not potentiate the responses induced by

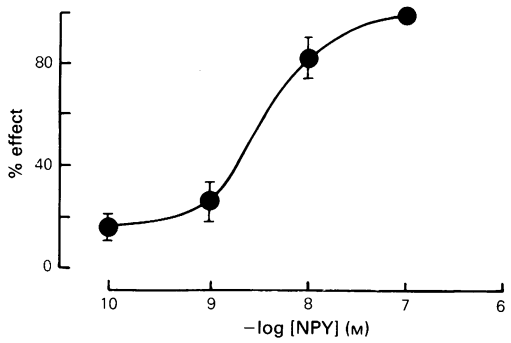

Figure 3 Concentration-response curve to neuropeptide Y (NPY, 10^{-10} to 10^{-7} M) in rat isolated mesenteric arterioles precontracted with 1.5×10^{-6} M phenylephrine ($n = 8$). Contraction is expressed as % of maximal contractile response of the vessels and the points show the mean with the bars representing s.e.mean.

either noradrenaline or phenylephrine. The pD_2 values of noradrenaline (5.8 ± 0.04, Figure 2a) and phenylephrine (5.3 ± 0.05, Figure 2b and 5.5 ± 0.04, Figure 2c) were not altered by NPY in the presence of either nitrendipine or diltiazem. Thus the components of the contractile responses of the vessels to the α-adrenoceptor agonists which were insensitive to nitrendipine or diltiazem were not affected by NPY.

Effect of NPY on vessels precontracted with phenylephrine

In order to study the relationship between the potentiation of the response to stimulation of α_1-adrenoceptors and the concentration of NPY, the peptide was added to vessels precontracted with 1.5×10^{-6} M phenylephrine. At this concentration, phenylephrine alone produced $30 \pm 6\%$ of the maximal contraction of the vessels. Further addition of NPY (10^{-10} to 10^{-7} M) increased tension in a concentration-dependent manner with a pD_2 value of 8.7 ± 0.1 ($n = 8$) as seen in Figure 3. The maximal tension reached in the presence of NPY was identical to the maximal tension induced by 10^{-5} M noradrenaline alone on the same arterioles.

Effects of NPY on relaxation induced by prazosin, forskolin, sodium nitroprusside or diltiazem in vessels precontracted with phenylephrine

The ability of NPY to reverse relaxation induced through different mechanisms was investigated on arterioles precontracted with phenylephrine and then relaxed by different drugs. The agonist was added at a single concentration, 3×10^{-6} M, which produced $78 \pm 6\%$ of the maximal response. In these conditions, further addition of 10^{-6} M nitrendipine or 10^{-5} M diltiazem completely relaxed the vessels, showing that only the calcium entry blocker-sensitive component of the response to phenylephrine was being studied. As shown in Figure 4, prazosin, forskolin and diltiazem relaxed the arterioles to the baseline tension with mean pD_2 values of 8.9 ± 0.1 ($n = 10$), 6.7 ± 0.1 ($n = 6$) and 6.7 ± 0.1 ($n = 6$), respectively. Sodium nitroprusside produced a maximal relaxation of $83 \pm 6\%$ with a pD_2 of 6.3 ± 0.1 ($n = 6$). For the following experiments, concentrations equal to the EC_{50} values were used to relax vessels precontracted with phenylephrine. The level of tension which was reached in these conditions was not significantly different from that obtained with 1.5×10^{-6} M phenylephrine alone. In addition, the level of tension did not differ significantly between preparations and was consistent over the period of the experiment.

Figure 5 shows the concentration-response curves to NPY on vessels precontracted with phenylephrine in the presence of the selected concentration of relaxing agent. NPY (10^{-10} to 10^{-7} M) increased tension up to the same maximum as

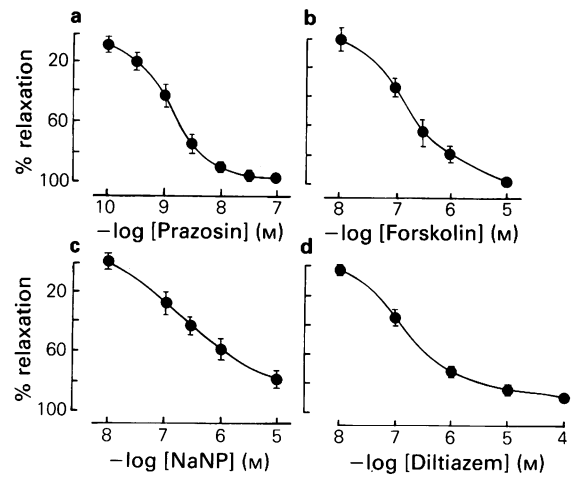

Figure 4 Relaxation induced by prazosin (a, $n = 10$), forskolin (b, $n = 6$), sodium nitroprusside (NaNP) (c, $n = 6$) and diltiazem (d, $n = 6$) in rat isolated mesenteric arterioles precontracted with 3×10^{-6} M phenylephrine. Relaxation is expressed as % of maximal relaxing response of the vessels to the baseline tension. Values are mean and vertical lines indicate s.e.mean.

obtained with 10^{-5} M noradrenaline, which represented the maximum contractile capacity of the vessels. The maximum contractions induced by NPY were approximately 20% higher than the precontractions induced by phenylephrine (3×10^{-6} M). The pD_2 values for NPY were respectively 8.4 ± 0.1 in the presence of prazosin ($n = 6$), 8.6 ± 0.2 in the presence of forskolin ($n = 6$), 8.7 ± 0.1 in the presence of sodium nitroprusside ($n = 6$), and 8.3 ± 0.1 in the presence of diltiazem ($n = 5$). These values, when compared by analysis of variance, were not significantly different from each other or from the pD_2 value of NPY obtained in the presence of 1.5×10^{-6} M phenylephrine alone.

Interaction between NPY and nitrendipine and between NPY and diltiazem on arterioles precontracted with phenylephrine

The concentration-response curve to nitrendipine was obtained cumulatively on vessels precontracted with 3×10^{-6} M phenylephrine since, unlike the other vasodilator agents, the dihydropyridine could not be removed between

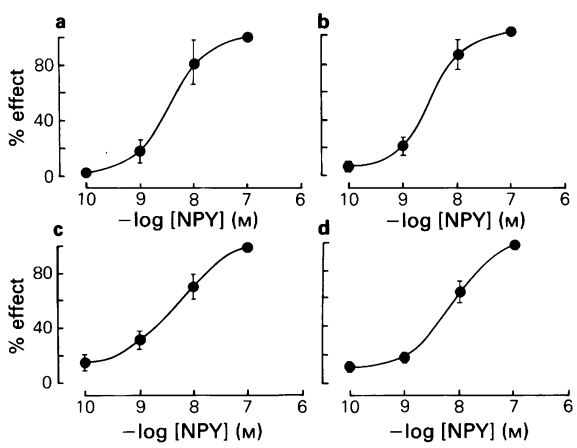

Figure 5 Effect of neuropeptide Y (NPY) on vessels precontracted with phenylephrine (3×10^{-6} M) in the presence of selected relaxing agents at a concentration equal to their respective EC_{50} values. Concentration-response curves to NPY in the presence of prazosin (a, $n = 6$), forskolin (b, $n = 6$), sodium nitroprusside (c, $n = 6$) and diltiazem (d, $n = 5$). The contraction is expressed as % of maximal contractile response of the vessels. The points are the mean with the error bars showing s.e.mean.

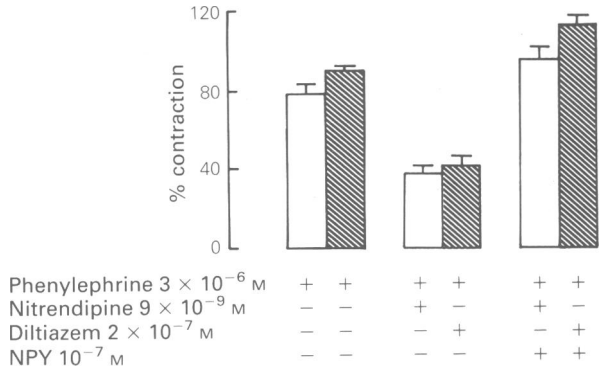

Figure 6 The effect of calcium antagonists, nitrendipine (9×10^{-9} M, open columns) and diltiazem (2×10^{-7} M, hatched columns) on contractions induced by 3×10^{-6} M phenylephrine and by subsequent addition of neuropeptide Y (NPY, 10^{-7} M) in rat mesenteric arterioles. Contractions are expressed as % of the contractile responses induced by 10^{-5} M noradrenaline which were 1331 ± 96 mg ($n = 7$) and 1662 ± 182 mg ($n = 5$), respectively with the experiments using nitrendipine or diltiazem as vasorelaxant agent. Each column represents the mean with the bars representing s.e.mean.

doses by washing. With this protocol, nitrendipine relaxed the arterioles to the baseline tension with mean pD_2 values of 9.0 ± 0.1 ($n = 6$).

For the following experiments, the protocol described in the Methods section was followed in order to compare the interaction between NPY and nitrendipine and between NPY and diltiazem. Only the maximal concentration of NPY (100 nM) was used in this experiment, since the concentration-response curve to NPY constructed non-cumulatively could not be obtained in the presence of nitrendipine due to the irreversibility of the action of dihydropyridine. Figure 6 shows that, when the contractile response of each vessel was expressed as % of maximal contraction with 10^{-5} M noradrenaline, the same level of contraction was obtained with 3×10^{-6} M phenylephrine plus 9×10^{-9} M nitrendipine and with 3×10^{-6} M phenylephrine plus 2×10^{-7} M diltiazem, which were $38 \pm 4\%$ ($n = 7$) and $42 \pm 5\%$ ($n = 5$), respectively. Subsequent addition of NPY (100 nM) increased tension up to the same maximum obtained with 10^{-5} M noradrenaline, both in the presence of phenylephrine plus 9×10^{-9} M nitrendipine ($95 \pm 7\%$) and in the presence of phenylephrine plus 2×10^{-7} M diltiazem ($113 \pm 5\%$), at a concentration at which the calcium entry blockers partially inhibited the influx of calcium induced by the agonist. Thus, no difference was observed in the interaction between NPY and nitrendipine and between NPY and diltiazem.

Discussion

The aim of the present work was to investigate the mechanism or mechanisms that might be implicated in the enhancement by NPY of the responses to α_1-adrenoceptor agonists in rat mesenteric arterioles. In these resistance arteries, NPY potentiates contractions elicited not only by the sympathetic neurotransmitter, noradrenaline, but also by other agonists and by addition of calcium to depolarized vessels (Andriantsitohaina & Stoclet, 1988a). Thus, the enhancement by NPY of the contractile response is not a receptor-specific mechanism and must involve a more general alteration of the pathway leading from smooth muscle excitation to contraction. Indeed, binding studies have not shown any change in the number or affinity of α-adrenoceptors in rat blood vessels (Pernow *et al.*, 1986), and only a slight increase in the number of α_2- (but not α_1) binding sites in rat medulla oblongata (Agnati *et al.*, 1983), under the influence of NPY.

The α-adrenoceptors of rat mesenteric arterioles have been described as being predominantly, if not exclusively, of the α_1 type (Cauvin & Malik, 1984). In accordance with this conclusion, we have found that the α_2-adrenoceptor agonists, clonidine and UK 14304, were unable to induce any contraction of the vessels. In addition, propranolol (3×10^{-6} M) did not alter the concentration-effect curves of noradrenaline, whether in the absence or in the presence of NPY (Andriantsitohaina & Stoclet, unpublished observations). Thus, the effects of noradrenaline and phenylephrine obtained in the present study did not involve any participation of β-adrenceptors.

The mechanisms thought to be implicated in the coupling between α_1-adrenoceptor activation and vascular smooth muscle cell contraction are the production of inositol 1,4,5-trisphosphate ($InsP_3$) and the influx of extracellular calcium through dihydropyridine-sensitive channels (see reviews: Exton, 1988; Minneman, 1988; Nichols & Ruffolo, 1988). The second pathway can be entirely inhibited in the presence of a dihydropyridine calcium entry blocker at a concentration which does not affect the production of $InsP_3$ (Han *et al.*, 1987). In the present study, we performed experiments with either nitrendipine at a maximally active concentration (10^{-6} M) or diltiazem (10^{-5} M), which belongs to another class of calcium entry blocker, at a concentration at which it blocked the dihydropyridine-sensitive component of the response to phenylephrine. Higher concentrations of diltiazem (10^{-4} M) decreased the maximal response to phenylephrine by about 80%, but this effect is probably due to an additional action, which depresses phenylephrine-contraction dependent on intracellular calcium release (Cauvin *et al.*, 1983; Saida & Van Breemen, 1983; Dacquet *et al.*, 1987). The results of the present work clearly demonstrate that, on rat mesenteric arterioles, NPY enhances the dihydropyridine-sensitive component of the response to α-adrenoceptor agonists, but does not affect the dihydropyridine-insensitive one.

An increase in α_1-adrenoceptor-induced accumulation of $InsP_3$ under the influence of NPY has been demonstrated in different regions of the rat brain and in rat vas deferens (Häggblad & Fredholm 1987; Hinson *et al.*, 1988), but was not found in rabbit cultured pulmonary artery cells (Reynolds & Yokota, 1988). In the latter cells, NPY did not elevate cytosolic calcium concentration. These results and those of the present work suggest that the mechanism by which NPY potentiates vasoconstriction induced by α_1-adrenoceptor stimulation might not involve the production of $InsP_3$ and subsequent release of intracellular calcium, even though NPY may act on the $InsP_3$ pathway in other tissues.

Another series of experiments was carried out to study further the potentiating effect of NPY on the calcium entry blocker-sensitive component of the response to stimulation of α_1-adrenoceptors. The experimental protocol was designed in order to obtain concentration-effect curves of NPY in various conditions. The peptide was added to arterioles brought to the same level of tension either by phenylephrine alone or by successive additions of a higher concentration of phenylephrine and of a relaxing agent at an appropriate concentration. Prazosin was used in order to displace partially the binding of phenylephrine from its sites, forskolin and sodium nitroprusside were used to increase adenylate cyclase (Seamon & Daly, 1986) or guanylate cyclase (Trembley *et al.*, 1988) activity respectively. Diltiazem and nitrendipine were used to block partially the influx of calcium. The contractile response induced by phenylephrine in these conditions was completely dependent on the entry of calcium through dihydropyridine- and diltiazem-sensitive channels, since it could be abolished by the addition of either of these agents. In all experimental conditions, NPY increased the arteriolar tension to that obtained with 10^{-5} M noradrenaline, which corresponds to the maximal contractile capacity of the vessels (further addition of other agonists or KCl did not produce any further increase in tension). It was found that the sensitivity of the vessels to NPY was not significantly different in the absence or in the presence of any of the relaxing agents and it was similar to sensitivities obtained for other isolated blood vessels (Edvinsson *et al.*, 1984a; Hanko *et al.*, 1986; Brayden & Conway, 1988; Suzuki *et al.*, 1988).

The extent of the potentiating effect of NPY seems to depend on the experimental protocol used. In the first series of

experiments, shown in Figure 2, 10^{-7} M NPY was added 5 min before the construction of a cumulative dose-response curve to the α_1-adrenoceptor agonist, whereas in subsequent experiments NPY was added when the response to phenylephrine alone or phenylephrine plus a vasorelaxant agent had reached equilibrium. In the latter case, the maximal response elicited by NPY was obtained rapidly (about 1 min), then the response decreased with time and returned to the level of precontraction in about 10 min. Thus, after 5 min preincubation, the effect of NPY may already be below its maximum and probably this time-dependent effect of NPY could explain why NPY was apparently less efficient in the first series of experiments.

As mentioned above, it has been shown in previous work that, in rat mesenteric arterioles, the potentiating effect of NPY is not receptor-specific and must involve a pathway which is common to coupling between stimulation of various receptors or depolarization and contraction (Andriantsitohaina & Stoclet, 1988a). In agreement with the view that NPY increases the efficiency of coupling between stimulation of α_1-adrenoceptors and contraction, the present results show no difference in the effect of NPY whether α_1-adrenoceptor stimulation was partial (phenylephrine alone or phenylephrine plus prazosin) or the response to submaximal stimulation of α_1-adrenoceptors was attenuated by forskolin, sodium nitroprusside, nitrendipine or diltiazem (the two latter at a concentration which was not sufficient to block completely the influx of calcium elicited by phenylephrine).

In rat mesenteric arterioles, a quantitative relationship has recently been demonstrated between the degree of stimulation of α_1-adrenoceptors and the intracellular concentration of free calcium ions (Bukoski *et al.*, 1989). In different vascular preparations, vasorelaxant agents which induce increases in cyclic AMP levels inhibit force production without reducing intracellular free calcium and those which increase cyclic GMP levels not only uncouple the calcium concentration-force relations but also decrease basal and vasoconstrictor-elevated cytosolic free calcium (Morgan & Morgan, 1984; Hassid, 1986; Paglin *et al.*, 1988). The results presented here do not support the contention that NPY affects the relationship between calcium concentration and active tension in rat mesenteric arterioles, since the peptide did not alter the dihydropyridine-insensitive component of the response to α_1-adrenoceptor agonists. They rather support the view that NPY increased calcium entry, in accordance with the fact that its effect was abolished in the presence of nitrendipine and diltiazem at concentrations which are able to block calcium influx entirely. This hypothesis is also supported by previous results showing that NPY potentiated contractions elicited by addition of $CaCl_2$ to KCl-depolarized rat isolated mesenteric arterioles and increased the sensitivity of these vessels to the calcium channel agonist Bay K 8644 (Andriantsitohaina & Stoclet, 1988a). Such a mechanism could also explain why NPY counteracted the relaxing effects of partial inhibition of calcium entry by nitrendipine and diltiazem at low concentrations, as well as those of drugs which decrease cytosolic free calcium concentration or calcium concentration and force relation.

Noradrenaline itself produces partial depolarization of rat mesenteric arterioles (Mulvany *et al.*, 1982). NPY might potentiate the neurotransmitter-induced vasoconstriction of these resistance vessels either by increasing depolarization and subsequent opening of voltage-dependent calcium channels, or by modulating calcium channel function through interaction with a coupling protein and/or the production of a second messenger. The evidence presented above does not support the hypothesis that increased $InsP_3$ is responsible for the potentiation by NPY of noradrenaline-induced vasoconstriction in rat mesenteric arterioles. However, it has to be noted that the analysis of data obtained by use of a mixture of three drugs in *in vitro* studies is complex. Nevertheless, the interpretations of the results given above open a new pathway for exploration of the mechanism of action of NPY. The intervention of a G protein and/or second messenger, subsequent to stimulation of NPY receptors, seems to be the more likely hypothesis.

The authors are indebted to Dr R. Hiley for fruitful discussions.

References

ABEL, P.W. & HAN, C. (1989). Effects of NPY on contraction, relaxation, and membrane potential of rabbit cerebral arteries. *J. Cardiovasc. Pharmacol.*, **13**, 52–63.

AGNATI, L.F., FUXE, K., BENFENATI, F., BATTISTINI, N., HÄRFSTRAND, A., TATEMOTO, K., HÖKFELT, T. & MUTT, V. (1983). Neuropeptide Y selectively increases the number of alpha$_2$-adrenergic binding sites in membranes of the medulla oblongata of the rat. *Acta Physiol. Scand.*, **118**, 293–295.

ALLEN, J.M. & BLOOM, S.R. (1986). Neuropeptide Y: a putative neurotransmitter. *Neurochem. Int.*, **8**, 1–8.

ANDRIANTSITOHAINA, R. & STOCLET, J.C. (1988a). Potentiation by neuropeptide Y of vasoconstriction in rat resistance arteries. *Br. J. Pharmacol.*, **95**, 219–228.

ANDRIANTSITOHAINA, R. & STOCLET, J.C. (1988b). Neuropeptide Y (NPY) counteracts relaxation of rat mesenteric arterioles induced by different mechanisms. *Br. J. Pharmacol.*, **96**, 219P.

BRAYDEN, J.E. & CONWAY, M.A. (1988). Neuropeptide Y and vasoactive intestinal polypeptide in cerebral arteries of the rat: relationship between innervation pattern and mechanical response. *Regul. Peptides*, **22**, 253–265.

BUKOSKI, R.D., BERGMANN, C., GAIRARD, A. & STOCLET, J.C. (1989). Intracellular Ca^{2+} and force determined simultaneously in isolated resistance arteries. *Am. J. Physiol.*, (in press).

CAUVIN, C., LOUTZENHISER, R. & VAN BREEMEN, C. (1983). Mechanism of calcium-induced vasodilation. *Ann. Rev. Pharmacol. Toxicol.*, **23**, 373–396.

CAUVIN, C. & MALIK, S. (1984). Induction of Ca^{++} influx and intracellular Ca^{++} release in isolated rat aorta and mesenteric alpha-1 receptors. *J. Pharmacol. Exp. Ther.*, **230**, 413–418.

DACQUET, C., MIRONNEAU, C. & MIRONNEAU, J. (1987). Effects of calcium entry blockers on calcium-dependent contractions of rat portal vein. *Br. J. Pharmacol.*, **92**, 203–211.

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.

EDVINSSON, L. (1985). Characterization of the contractile effect of neuropeptide Y in feline cerebral arteries. *Acta Physiol. Scand.*, **125**, 33–41.

EDVINSSON, L., EMSON, P., McCULLOCH, J., TATEMOTO, K. & UDDMAN, R. (1984a). Neuropeptide Y: Immunocytochemical localization and effect upon feline pial arteries and veins *in vitro* and *in situ*. *Acta Physiol. Scand.*, **122**, 155–163.

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

EDVINSSON, L., HÄKANSON, R., WAHLESTEDT, C. & UDDMAN, R. (1987). Effects of neuropeptide Y on the cardiovascular system. *Trends Pharmacol. Sci.*, **8**, 231–235.

EKBLAD, E., EDVINSSON, L., WAHLESTEDT, C., UDDMAN, R., HÄKANSON, R. & SUNDLER, F. (1984). Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibers. *Regul. Peptides*, **8**, 225–235.

EXTON, J.H. (1988). Mechanism of calcium-mobilizing agonists: some variations on a young theme. *FASEB J.*, **2**, 2670–2676.

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.

GLOVER, W.E. (1985). Increased sensitivity of rabbit ear artery to noradrenaline following perivascular nerve stimulation may be a response to neuropeptide Y released as cotransmitter. *Clin. Exp. Pharmacol. Physiol.*, **12**, 227–230.

GRAY, T.S. & MORLEY, J.E. (1986). Neuropeptide Y: anatomical distribution and possible function in mammalian nervous system. *Life Sci.*, **38**, 389–401.

HÄGGBLAD, J. & FREDHOLM, B.B. (1987). Adenosine and neuropeptide Y enhance α_1-adrenoceptor-induced accumulation of ino-

sitol phosphates and attenuate forskolin-induced accumulation of cyclic AMP in rat vas deferens. *Neurosci. Lett.*, **82**, 211–216.

HAN, C. & ABEL, P.W. (1987). Neuropeptide Y potentiates contraction and inhibits relaxation of rabbit coronary arteries. *J. Cardiovasc. Pharmacol.*, **9**, 675–681.

HAN, C., ABEL, P.W. & MINNEMAN, K.P. (1987). α_1-adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca^{2+} in smooth muscle. *Nature*, **329**, 333–335.

HANKO, J.H., TÖRNEBRANDT, K., HARDEBO, J.E., HÄHRSTRÖM, J., NOBIN, A. & OWMAN, C. (1986). Neuropeptide Y induces and modulates vasoconstriction in intracranial and peripheral vessels of animals and man. *J. Auton. Pharmacol.*, **6**, 117–124.

HASSID, A. (1986). Atriopeptin II decreases cytosolic free Ca in cultured vascular smooth muscle cells. *Am. J. Physiol.*, **251**, C681–C686.

HINSON, J., RAUH, C. & COUPET, J. (1988). Neuropeptide Y stimulates inositol phospholipid hydrolysis in rat brain miniprisms. *Brain Res.*, **446**, 379–382.

JUAN, H., SAMETZ, W., SARIA, A. & PÖCH, G. (1988). Eicosapentaenoic acid inhibits vasoconstrictor- and noradrenaline-potentiating effects of neuropeptide Y in the isolated rabbit ear. *J. Auton. Nerv. Syst.*, **22**, 237–242.

JULOU, G. & FRESLON, J.L. (1986). Effects of calcium entry blockers Ca^{2+}-induced contraction of depolarized and noradrenaline-exposed rat resistance vessels. *Eur. J. Pharmacol.*, **129**, 261–270.

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

LUNDBERG, J.M., TERENIUS, L., HÖKFELT, T., MARTLING, R., TATEMOTO, K., MUTT, V., POLAK, J., BLOOM, S. & GOLDSTEIN, M. (1982). Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. *Acta Physiol. Scand.*, **116**, 477–480.

LUNDBERG, J.M., PERNOW, J., FRANCO-CERECEDA, A. & RUDEHILL, A. (1987). Effects of antihypertensive drugs on sympathetic vascular control in relation to neuropeptide Y. *J. Cardiovasc. Pharmacol.*, **10**, S51–S68.

LUNDBERG, J.M., HEMSEN, A., LARSSON, O., RUDEHILL, A., SARIA, A. & FREDHOLM B.B. (1988). Neuropeptide Y receptor in pig spleen: binding characteristics, reduction of cyclic AMP formation and calcium inhibition of vasoconstriction. *Eur. J. Pharmacol.*, **145**, 21–30.

MINNEMAN, K.P. (1988). α_1-adrenergic receptor subtypes, inositol phosphates, and sources of cell Ca^{2+}. *Pharmacol. Rev.*, **40**, 87–120.

MORGAN, J.P. & MORGAN, K.G. (1984). Alteration of cytoplasmic ionized calcium levels in smooth muscle by vasodilators in the ferret. *J. Physiol.*, **357**, 539–551.

MULVANY, M.J. & HALPERN, W. (1977). Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ. Res.*, **41**, 19–26.

MULVANY, M.J., NILSSON, H. & FLATMAN, J.A. (1982). Role of membrane potential in the response of rat small mesenteric arteries to exogenous noradrenaline stimulation. *J. Physiol.*, **332**, 363–373.

NEILD, T.O. (1987). Actions of neuropeptide Y on innervated and denervated rat tail artery. *J. Physiol.*, **386**, 19–30.

NEILD, T.O. (1988). Actions of neuropeptide Y on arterial and arteriolar smooth muscle. In *Resistance Arteries* ed. Halpern, W., Pegrian, B.L., Gravden, J.E., Makey, K., McLaughlin, M.K. & Osol, G. pp. 385–389. Ithaca, N.Y. Perianatology Press.

NICHOLS, A.J. & RUFFOLO, R.R. (1988). The relationship of α-adrenoceptor reserve and agonist intrinsic efficacy to calcium utilization in the vasculature. *Trends Pharmacol. Sci.*, **8**, 236–241.

O'DONOHUE, T.L., CHRONWALL, R.M., PRUSS, R.M., MEZEY, E., KISS, J.Z., EIDEN, L.E., MASSARI, V.J., TESSEL, R.E., PICKEL, V.M., DIMAGGIO, D.A., HOTCHKISS, A.J., CROWLEY, W.R. & ZUKOWSKA-GROJEC, Z. (1985). Neuropeptide Y and peptide YY neuronal and endocrine systems. *Peptides*, **6**, 755–768.

PAGLIN, S., TAKUWA, Y., KAMM, K.E., STULL, J.T., GAVRAS, H. & RASMUSSEN, H. (1988). Atrial natriuretic peptide inhibits the agonist-induced increase in extent of myosin light chain phosphorylation in aortic smooth muscle. *J. Biol. Chem.*, **263**, 13117–13120.

PEGRAM, B.L. & HUNTER, J.M. (1988). Hemodynamic effects of neuropeptide Y in rats. In *Resistance Arteries* ed. Halpern, W., Pegrian, B.L., Gravden, J.E., Makey, K., McLaughlin, M.K. & Osol, G. pp. 80–84. Ithaca, N.Y. Perianatology Press.

PEGRAM, B.L. & HUNTER, J.M. (1988). Hemodynamic effects of neuropeptide Y in rats. In *Resistance Arteries* ed. Halpern, W., Pegrian, B.L., Gravden, J.E., Makey, K., McLaughlin, M.K. & Osol, G. pp. 80–84. Ithaca, N.Y. Perianatology Press.

POTTER, E.K. (1988). Neuropeptide Y as an autonomic neurotransmitter. *Pharmacol. Ther.*, **37**, 251–273.

REYNOLDS, E.E. & YOKOTA, S. (1988). Neuropeptide Y receptor-effector coupling mechanisms in cultured vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.*, **151**, 919–925.

SAÏDA, K. & VAN BREEMEN, C. (1983). Mechanism of Ca^{++} antagonist-induced vasodilation: intracellular action. *Circ. Res.*, **52**, 137–142.

SEAMON, K.B. & DALY, J.W. (1986). Forskolin: its biological and chemical properties. *Adv. Cyclic Nucleotide Prot. Phosphoryl. Res.*, **20**, 1–150.

SUNDLER, F., HÄKANSON, R., EKBLAD, E., UDDMAN, R. & WAHLESTEDT, C. (1986). Neuropeptide Y in the peripheral adrenergic and enteric nervous systems. *Int. Rev. Cyt.*, **102**, 243–269.

SUZUKI, Y., SHIBUYA, M., IKEGAKI, I., SATOH, S., TAKAYASU, M. & ASANO, T. (1988). Effects of neuropeptide Y on canine cerebral circulation. *Eur. J. Pharmacol.*, **146**, 271–277.

TREMBLEY, J., GERZER, R. & HAMET, P. (1988). Cyclic GMP in cell function. *Adv. Second Messenger Phosphoprot. Res.*, **22**, 319–383.

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

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

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.

ZUKOWSKA-GROJEC, Z., MARKS, E.S. & HAASS, M. (1987). Neuropeptide Y is a potent vasoconstrictor and cardiodepressant in rat. *Am. J. Physiol.*, **253**, H1234–H1239.

(Received July 17, 1989
Revised September 27, 1989
Accepted October 5, 1989)