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Characterization of vascular neuropeptide Y receptors

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1 In the present study we compared neuropeptide Y (NPY) and NPY-related analogues for their ability to activate or bind to vascular NPY receptors in four experimental set-ups. Previous results have suggested the existence of different receptor subtypes, Y_1 receptors requiring full-length NPY (1–36) or [Pro^{34}]-NPY, and Y_2 receptors recognizing also N-terminally truncated forms of NPY but not [Pro^{34}]-NPY.

2 NPY 1–36 and [Pro^{34}]-NPY dose-dependently increased arterial pressure in the anaesthetized rat with a similar magnitude and potency. NPY 2–36 was much less potent than NPY 1–36. NPY 4–36 and NPY 11–36 were inactive even at a dose as high as 10 nmol kg^{-1}.

3 NPY 1–36, [Pro^{34}]-NPY, NPY 2–36 and NPY 5–36 concentration-dependently increased the coronary resistance in the Langendorff preparation of the rat. NPY 1–36 and [Pro^{34}]-NPY were equipotent, while NPY 2–36 and NPY 5–36 were about 7 and 20 times less potent. At 0.3 μM, NPY 11–36, NPY 20–36 and NPY 22–36 induced a slight contraction while NPY 23–36 was inactive.

4 NPY 1–36, [Pro^{34}]-NPY, NPY 2–36, NPY 4–36, NPY 5–36 and NPY 11–36 evoked concentration-dependent contractions in the isolated inferior caval vein of the rat and guinea-pig. [Pro^{34}]-NPY was more potent than NPY 1–36. NPY 2–36 was equipotent with NPY 1–36, while NPY 4–36, NPY 5–36 and NPY 11–36 were approximately 30 times less potent.

5 [Pro^{34}]-NPY was equipotent with NPY 1–36 in displacing the ^{125}I-labelled gut hormone peptide ([^{125}I]-PYY) from rat aortic smooth muscle cells, while NPY 2–36 and shorter forms of NPY were much less potent or inactive.

6 In caval vein smooth muscle cells of the rat, the displacement pattern was more complex than in aortic smooth muscle cells, in that both [Pro^{34}]-NPY and NPY 13–36 effectively displaced the radioligand, albeit none of them completely.

7 In conclusion, the NPY-evoked pressor response in the whole rat and coronary vessels seems to be mediated by vascular Y_1 receptors and the binding characteristics of the NPY-related peptides in the aortic smooth muscle cells correspond to a population of such receptors. In the caval vein, the profile of the bioactivity and the binding affinity of the NPY-related peptides suggest a mixed population of Y_1/Y_2 receptors.

Keywords: Hypertension; vasoconstriction; coronary resistance; radioligand binding; neuropeptide Y (NPY); Y_1-receptors; Y_2-receptors

Introduction

Neuropeptide Y (NPY) has a wide distribution in the peripheral and central nervous system. It seems to be co-localized with noradrenaline (NA) in cardiovascular sympathetic fibres (Lundberg *et al.*, 1982b; Ekblad *et al.*, 1984; Uddman *et al.*, 1985; Sundler *et al.*, 1986). The cardiovascular effects of NPY have been analyzed in some detail during the last few years. Intravenous injection of NPY raises the arterial pressure (AP) (Lundberg *et al.*, 1982b; Grundemar *et al.*, 1990) but this apparently simple response is the net result of a complex mode of action. Briefly, NPY may exert three effects at sympathetic neuroeffector junctions: (1) a direct postjunctional response (vasoconstriction); (2) a postjunctional potentiation of the response to vasoconstrictors, such as NA; and (3) a prejunctional suppression of stimulated NA release (Lundberg & Stjärne, 1984; Håkanson *et al.*, 1986; Wahlestedt *et al.*, 1986; 1990b). There is much evidence to suggest that NPY acts on two receptor subtypes, tentatively referred to as Y_1 and Y_2 (for reviews see Wahlestedt *et al.*, 1990a; Schwartz *et al.*, 1990). The Y_1-type receptor seems to be located postjunctionally and is able to evoke vasoconstriction, while the Y_2-type receptor seems to be predominantly prejunctional, suppressing the stimulated release of NA. The Y_2-type receptor recognizes not only the parent molecule but also truncated C-terminal fragments of NPY (Wahlestedt *et al.*, 1986). In fact, on the latter receptor NPY 13–36 is only slightly less potent than NPY 1–36; a progressive N-terminal shortening of the molecule results in a gradual loss of potency (Grundemar & Håkanson, 1990). The Y_1-type receptor seems to require the whole NPY molecule in order to become fully activated since C-terminal fragments are inactive or much less potent (Rioux *et al.*, 1986; Wahlestedt *et al.*, 1986). [Pro^{34}]-NPY is a recently developed analogue that acts as a selective Y_1-type receptor agonist (Krstenansky *et al.*, 1990; Dumont *et al.*, 1990). The gut hormone peptide YY (PYY) displays 70% homology with NPY and is about equipotent with NPY on either receptor subtype (Lundberg *et al.*, 1982a; Wahlestedt *et al.*, 1986; Grundemar & Håkanson, 1990). Another homologous peptide hormone, pancreatic polypeptide (PP), has virtually no affinity for either receptor subtype (Schwartz *et al.*, 1990). Supporting evidence for the Y_1/Y_2 receptor subtype concept has come from binding studies of neuroblastoma cell lines that seem to express either Y_1 or Y_2 receptors (Sheikh *et al.*, 1989; Schwartz *et al.*, 1990).

In the present study we compared NPY 1–36, C-terminal fragments of NPY and the Y_1-type receptor selective analogue [Pro^{34}]-NPY for their ability to evoke vasoconstriction or bind to vascular NPY receptors. In four different experimental set-ups we measured: (1) AP in the anaesthetized rat, (2) coronary resistance in the Langendorff preparation of the rat heart, (3) contractile effects in guinea-pig isolated inferior

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caval vein, and (4) binding of NPY 1–36 and NPY-related peptides to cultured rat aortic and caval vein smooth muscle cells.

Methods

Arterial blood pressure

Male Sprague-Dawley rats (200–250 g) were anaesthetized with intraperitoneal administration of 0.4–0.5 ml ketamine (50 mg ml^{-1}) (Ketalar, Parke-Davis) and 0.2–0.25 ml xylazine (20 mg ml^{-1}) (Rompun vet, Bayer). The left jugular vein and right femoral artery were cannulated with polyethylene catheters for i.v. administration of the agents and for continuous recording of systemic blood pressure via a Statham P23 pressure transducer to a Grass model 7 polygraph. The histamine H_1-receptor antagonist, mepyramine, was injected (10 mg kg^{-1} i.v.) in order to prevent the effects of NPY-evoked histamine release (Grundemar *et al.*, 1990) and experimentation was started (usually 10 min later) when the AP had stabilized at 100–120 mmHg. The agents were injected slowly (approx. 20 s) in a volume of 100 μl (room temperature), followed by washing the catheter with 100 μl saline. Dose-response curves were constructed from experiments in which single doses of each peptide were administered to each rat.

Perfused Langendorff

Male Sprague-Dawley rats (250–350 g) were killed, the chest was opened, the heart and lungs were excised and placed in Krebs solution containing 10 units ml^{-1} heparin (room temperature). The lungs, connective tissue and large vessels were removed from the heart to expose the aortic arch which was cannulated retrogradely to allow perfusion of the coronary circulation by the Langendorff technique (Vleeming *et al.*, 1989). Perfusion was performed at a constant pressure of 80 cm water. The perfusion fluid consisted of Krebs solution of the following composition (in mM): NaCl 100, KCl 4.0, $MgSO_4$ 1.5, $CaCl_2$ 2.0, $NaHCO_3$ 20.0, NaH_2PO_4 1.5, Na-acetate 20.0 and D-glucose 10.0; maintained at 33°C, pH 7.4 and equilibrated with 95% O_2 and 5% CO_2. Coronary flow was measured by determination of the volume of perfusate through the isolated heart per unit time. The results are presented as coronary resistance, i.e. the inverted value of the coronary flow. Before addition of peptides the coronary flow was about 3 ml min^{-1}. Peptides were administered by changing the Krebs solution to one containing Krebs plus the appropriate concentration of each peptide. Each preparation was exposed to only 1 or 2 concentrations of each peptide (at least 20 min interval).

Isolated vessels

Sprague-Dawley rats (200–250 g) and guinea-pigs (Dunklin-Hartley, 350–450 g) were killed by a blow on the head and exsanguinated. The proximal part of the inferior caval vein was removed close to the right auricle. The vessel was divided into three circular segments (2 mm wide) which were transferred to a thermostated bath (37°C), containing Krebs solution of the following composition (in mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 15.0, NaH_2PO_4 1.2 and glucose 6.0. The solution was bubbled continuously with 95% O_2 and 5% CO_2, giving a pH of 7.4. The vascular segments were mounted on two L-shaped stainless steel wires (0.1 mm ø), one of which was connected to a force-displacement transducer (Grass Instrument FT 03C) for continuous tension recording on a Grass polygraph (Högestätt *et al.*, 1983). The resting tension was gradually adjusted to 2 mN. The contractile capacity was examined by adding an isotonic 60 mM potassium Krebs solution (NaCl replaced by KCl); these contractions served as an internal control. After wash-out of the potassium-enriched solution, increasing doses of the different peptides were added to each bath in a cumlative manner (at least 3 min interval). Each segment was exposed to one peptide only. Contractile responses were expressed in relation to the potassium-evoked contraction.

Cultured rat aortic and caval vein smooth muscle cells

The aorta and the inferior caval vein were taken out from male Sprague-Dawley rats under sterile conditions and primary cultures of smooth muscle cells were prepared essentially as described by Meyer-Lehnert & Schrier (1989). Thus, the vessels were collagenase-treated (2 mg ml^{-1}) in Eagle's minimum essential medium, followed by removal of the adventitia and outer media. The vessels were then cut longitudinally and the intima scraped off. Following mincing and further collagenase treatment (3 h) with continuous stirring, the material was triturated 20 times through 18 and 16 gauge needles. The resulting single-cell suspension was centrifuged (1000 r.p.m.; 5 min) and resuspended in an incubation medium containing 10% foetal calf serum (GIBCO). This was repeated three times. Cells were plated on 6-well plates (35 × 35 mm) for the binding experiments (10^6 smooth muscle cells/well) after 8 passages. In parallel experiments, cell viability was tested (exclusion of trypan blue). Routinely, the cells were inspected with phase-contrast microscopy. The cells were defined as smooth muscle cells if stained for actin and desmin by immunocytochemistry (Chamley-Campbell *et al.*, 1979). Binding of [^{125}I]-PYY (New England Nuclear) was tested as previously described (Walker & Miller, 1988; Wahlestedt *et al.*, 1990a). Briefly, intact smooth muscle cells were washed three times in buffer of the following composition (in mM): NaCl 137, KCl 5.4, KH_2PO_4 0.44, $CaCl_2$ 1.25, $MgSO_4$ 0.81, HEPES 20, 0.1% bacitracin and 0.3% bovine serum albumin. The cells were incubated in 1 ml buffer with [^{125}I]-PYY (100 pmol) for 60 min at 37°C with or without various concentrations of competing non-labelled peptides. Binding reaction was interrupted by washing the cells four times in ice-cold buffer and the cells were then exposed to lysis buffer (3 M acetic acid with 8 M urea and 2%, v/v, Nonidet P-40; 1 + 0.5 ml). The cell membrane fragments were transferred to test tubes and the radioactivity counted. All binding experiments were performed in triplicate. Specific binding was defined in the presence of 1 μM unlabelled NPY 1–36 and was approximately 50 and 65% of total binding in smooth muscle cells from aorta and caval vein, respectively.

NPY and analogues: NPY 1–36 (porcine), NPY 20–36 and NPY 22–36 were obtained from Peninsula, Merseyside, St Helens, UK. NPY 2–36 and NPY 5–36 were purchased from Richelieu Biotech, QC, Canada. [Pro^{34}]-NPY and NPY 4–36 were kind gifts from Prof. T.W. Schwartz, Copenhagen, Denmark. NPY 11–36 and NPY 23–36 were synthesized by solid-phase synthesis and purified to at least 96% by h.p.l.c. (W. Krzeminsky, Ferring AB, Malmö, Sweden and H. Franzén, Dept of Med. Chem, Univ. of Lund, Lund, Sweden, respectively). NPY 13–36 and NPY 18–36 were kind gifts from Dr A. Fournier, Canada. All NPY-related peptides were based on porcine NPY.

Statistics

The pharmacological response to each peptide was characterized by the maximum response (E_{max}) and the pD_2 values (the negative logarithm of the dose or molar concentration of the peptide that elicits 50% of the maximum response). In the binding studies the potency with which each peptide displaced the radiolabelled ligand was expressed as pIC_{50} (the negative logarithm of the molar concentration of the peptide that displaces 50% of the ligand). These parameters were estimated by linear regression analysis of the results in the 10–90% response interval. Statistical analysis of the pD_2 or pIC_{50} values for the different peptides was performed by means of unpaired Student's t test. Data are presented as means ± s.e.means.

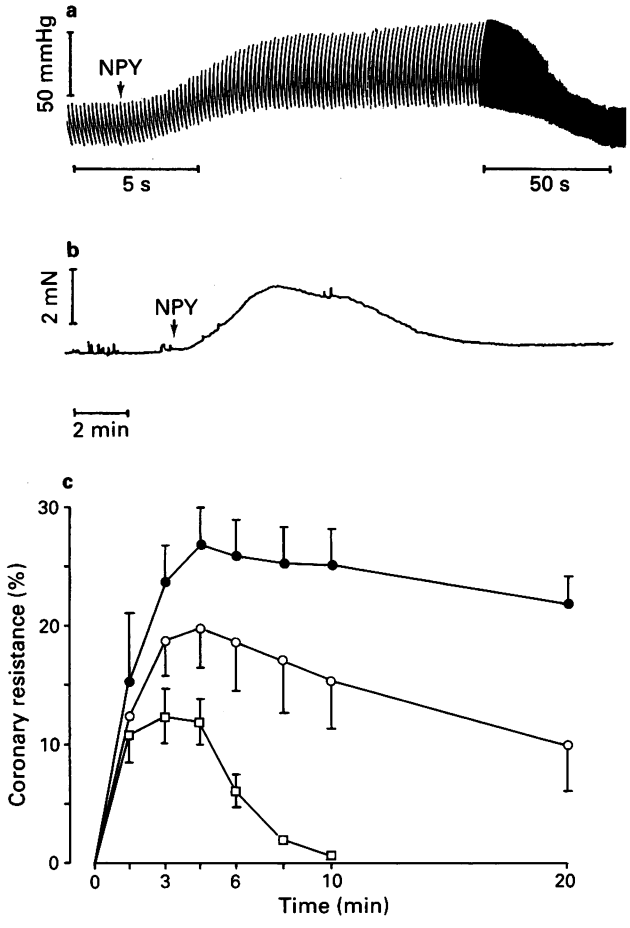

Figure 1 Tracings showing effects on arterial pressure (AP) of neuropeptide Y (NPY, 10 nmol kg^{-1}) injected i.v. into an anaesthetized rat (a), vasoconstriction evoked by NPY (0.1 μM) in the guinea-pig isolated inferior caval vein (b) and time course of the increase in coronary resistance in the perfused rat Langendorff preparation evoked by 5 nmol NPY 1–36 (●), NPY 11–36 (○) or NPY 22–36 (□) (c). See text for further information. Each value is the mean of 4–6 experiments. Vertical bars give s.e.mean.

Results

Pressor effects in the anaesthetized rat

In rats pretreated with the histamine H_1-receptor antagonist, mepyramine, i.v. injection of NPY 1–36 or [Pro^{34}]-NPY (0.1–10 nmol kg^{-1}) raised the AP dose-dependently (Figures 1 and 2). The responses were of a similar magnitude and potency; the pD_2 values were 9.06 ± 0.25 and 9.19 ± 0.38, respectively (expressed as bolus dose in neg log mol kg^{-1}). At 10 nmol kg^{-1} the pressor response to NPY 1–36 lasted for 1–2 min (Figure 1). The heart rate (HR) was not affected. NPY 2–36 was much less potent than NPY 1–36 (Figure 2; Table 1). NPY 4–36 (n = 4) and NPY 11–36 (n = 3) were inactive at 10 nmol kg^{-1}.

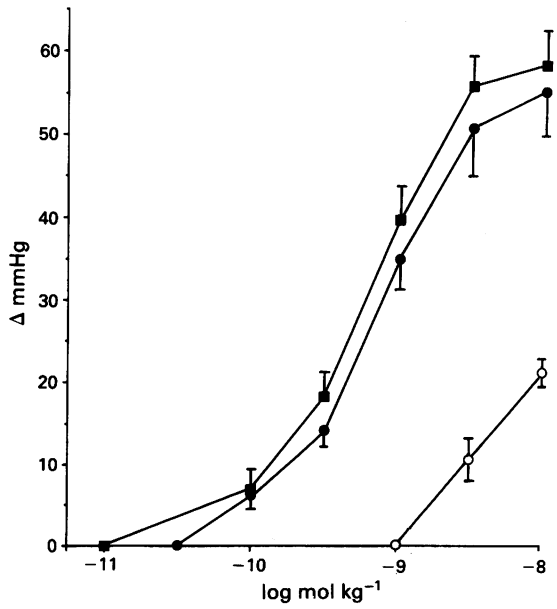

Figure 2 Dose-response curves show the change in arterial pressure (AP) evoked by NPY 1–36 (●), [Pro^{34}]-NPY (■) and NPY 2–36 (○) in the anaesthetized rat. The negative log ED_{50} values for NPY 1–36 and [Pro^{34}]-NPY were 9.06 ± 0.25 and 9.19 ± 0.38, respectively. n = 4–5 for each dose and peptide.

Increased coronary resistance in the perfused Langendorff heart

NPY 1–36, [Pro^{34}]-NPY, NPY 2–36 and NPY 5–36 concentration-dependently increased the coronary resistance (Figure 3). NPY 1–36 and [Pro^{34}]-NPY were approx. equipotent; the pD_2 values were 8.79 ± 0.24 and 8.50 ± 0.27, respectively (expressed as neg log molar concentration). NPY 2–36 and NPY 5–36 were less potent than NPY 1–36 with pD_2 values of 7.97 ± 0.27 ($P < 0.05$) and 7.46 ± 0.23 ($P < 0.05$), respectively (Figure 3; Table 1). NPY 11–36, NPY 20–36 and NPY 22–36 induced a slight increase in coronary resistance at the highest concentration tested (0.3 μM) (Figure 3), while NPY 23–36 was without effect (n = 4). In a series of experiments 5 nmol NPY 1–36, NPY 11–36 or NPY 22–36 were injected into the catheters supplying the coronary vessels with perfused Krebs solution. It was shown that the maximum response was elicited after about 4–5 min and that the duration of the effect was reduced with progressive shortening of the moleculte (Figure 1).

Contractile responses of the isolated inferior caval vein

NPY 1–36 elicited concentration-dependent contractions of the rat and guinea-pig caval vein. However, since the NPY-evoked responses were greater in the guinea-pig caval vein, these vessels were used in the following series of experiments.

NPY 1–36, [Pro^{34}]-NPY, NPY 2–36, NPY 4–36, NPY 5–36 and NPY 11–36 evoked concentration-dependent contractions (Figure 4). The pD_2 and E_{max} values are shown in

Table 1 The rank order, in terms of pharmacological effect on or binding affinity to the vascular neuropeptide Y (NPY) receptor in five experimental set-ups

	Pharmacological effect
Arterial pressure	[Pro^{34}]-NPY = NPY 1–36 ⩾ NPY 2–36; NPY 4–36 (inactive at 10 nmol kg^{-1})
Coronary resistance	[Pro^{34}]-NPY = NPY 1–36 > NPY 2–36 ⩾ NPY 5–36 > NPY 11–36
Vasoconstriction	[Pro^{34}]-NPY > NPY 1–36 = NPY 2–36 ⩾ NPY 4–36 = NPY 5–36 > NPY 11–36
	Binding to vascular smooth muscle cells
Binding to aorta	[Pro^{34}]-NPY = NPY 1–36 ⩾ NPY 2–36 > NPY 11–36 = NPY 13–36 > NPY 18–36
Binding to caval vein	[Pro^{34}]-NPY = NPY 1–36 > NPY 13–36

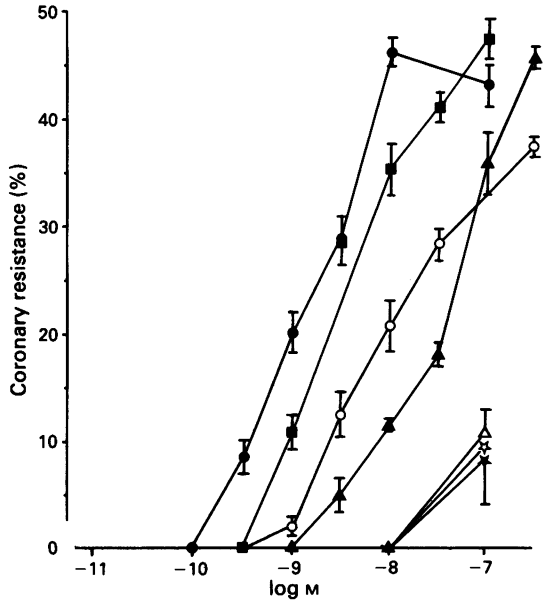

Figure 3 Concentration-response curves showing effects of NPY 1–36 (●), [Pro^{34}]-NPY (■), NPY 2–36 (○), NPY 5–36 (▲), NPY 11–36 (△), NPY 20–36 (★) and NPY 22–36 (☆) on the coronary resistance in the Langendorff perfused heart of the rat. E_{max} for [Pro^{34}]-NPY and NPY 5–36 were assumed to be the same as the E_{max} for NPY 1–36 and NPY 2–36. $n = 4$–6 for each concentration and peptide.

Table 2. [Pro^{34}]-NPY was approximately three times more potent than NPY 1–36 ($P < 0.05$). NPY 2–36 was about equipotent with NPY 1–36, whereas NPY 4–36, NPY 5–36 and NPY 11–36 were about 30 times less potent than NPY 1–36 (Table 1). Since the concentration-response curves for the latter peptides did not reach maximum, the respective pD_2 and E_{max} values could not be calculated. The onset of the vasoconstriction by NPY and related peptides was gradual, reached maximum after several minutes and relaxed spontaneously (Figure 1).

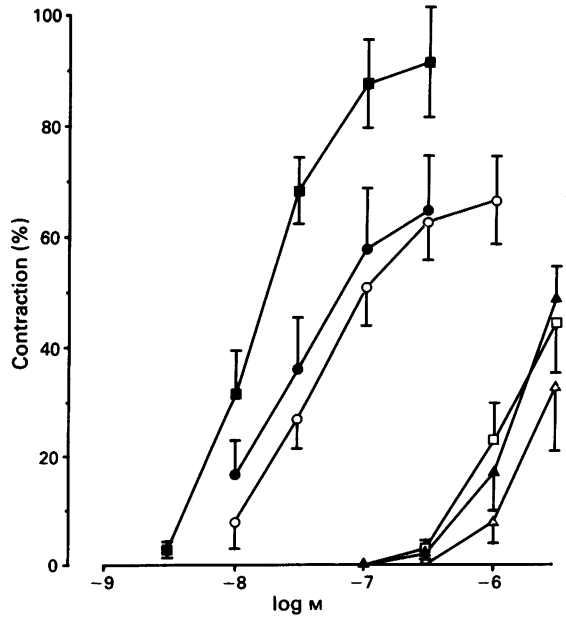

Figure 4 Contractile responses to NPY 1–36 ($n = 9$) (●),[Pro^{34}]-NPY ($n = 8$) (■), NPY 2–36 ($n = 10$) (○), NPY 4–36 ($n = 7$) (□), NPY 5–36 ($n = 5$) (▲) and NPY 11–36 ($n = 4$) (△) in the guinea-pig isolated inferior caval vein. Responses are expressed as a percentage of the 60 mM potassium-induced contraction.

Table 2 Ability of neuropeptide Y (NPY) and various analogues to contract the inferior caval vein of the guinea-pig

	pD_2	E_{max} (%)	n
NPY 1–36	7.5 ± 0.2	65 ± 10	9
[Pro^{34}]-NPY	8.0 ± 0.1*	92 ± 10	8
NPY 2–36	7.3 ± 0.1	67 ± 8	10
NPY 4–36	(+)		7
NPY 5–36	(+)		5
NPY 11–36	(+)		4

Values are means ± s.e.mean.
* Statistically different from NPY 1–36. (+), the peptide induced a contraction at ⩾0.3 μM only. (%), contraction as a percentage of the potassium-evoked contraction. *n*, the number of animals.

Binding to aortic and caval vein smooth muscle cells

NPY binding sites can be labelled by [^{125}I]-PYY (e.g. Walker & Miller, 1988). PYY is known to have qualitatively the same vascular effects as NPY (e.g. Wahlestedt *et al.*, 1990a), therefore [^{125}I]-PYY was used in this study. Displacement curves were generated by co-incubation with unlabelled peptides in increasing concentrations.

Displacement curves for NPY 1–36, [Pro^{34}]-NPY and NPY 13–36 in aortic smooth muscle cells (passage 8) are shown in Figure 5. It can be seen that NPY 13–36 is much less potent (2–3 orders of magnitude) than the other two peptides. The following pIC_{50} values were calculated: NPY 1–36,

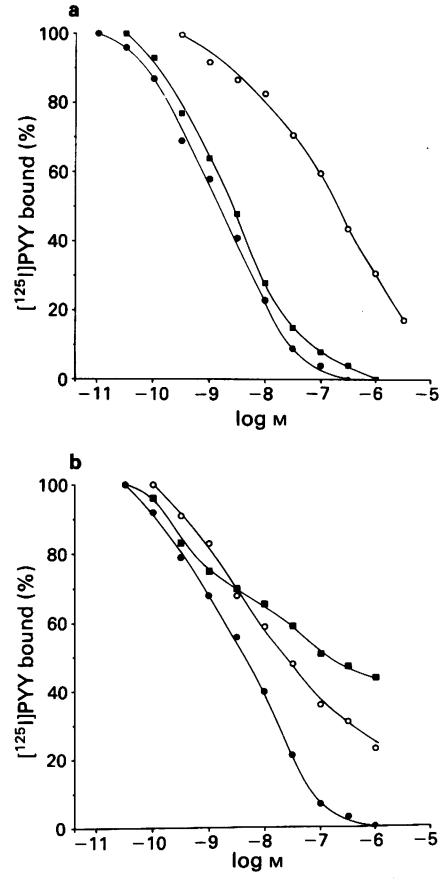

Figure 5 Displacement of [^{125}I]-PYY from (a) rat aortic or (b) inferior caval vein smooth muscle cells with NPY 1–36 (●), [Pro^{34}]-NPY (■) and NPY 13–36 (○). Each experiment was done in triplicate and repeated three times. For clarity only the mean values are indicated.

8.89 ± 0.14; [Pro^{34}]-NPY, 8.66 ± 0.14; NPY 2–36, 7.21 ± 0.11; NPY 11–36, 6.93 ± 0.09; NPY 13–36, 6.72 ± 0.13; NPY 18–36, 6.19 ± 0.20 (n = 3–5 for each peptide) (Table 1). These values differ slightly from those reported by Shen *et al.* (1990), who used cells from passage 2 or 3. Displacement occurred in a monophasic pattern, indicating the presence of a homogeneous population of binding sites displaying the binding characteristics of a Y_1-type receptor (e.g. see Wahlestedt *et al.*, 1990a).

In the caval vein smooth muscle cells, the displacement pattern was more complex, conforming to a mixed rather than single receptor population. As can be seen in Figure 5b, the curves could not be used to calculate useful pIC_{50} values. Both the Y_1-receptor-selective agonist [Pro^{34}]-NPY and the Y_2-receptor selective agonist NPY 13–36, potently, albeit incompletely, displaced [^{125}I]-PYY.

Discussion

A number of authors have shown that NPY is a powerful vasoconstrictor *in vivo* (e.g. Maturi *et al.*, 1989). Thus, i.v. injection of NPY evokes a dose-dependent increase in AP (Lundberg *et al.*, 1982b; Grundemar *et al.*, 1990). High doses of NPY evoke an initial rise followed by a long-lasting fall in AP that can be prevented by pretreatment with histamine H_1-receptor antagonists or with the histamine liberator compound 48/80, indicating that the fall in AP can be attributed to histamine release from mast cells (Grundemar *et al.*, 1990). The depressor response can be reproduced by high doses of different C-terminal NPY fragments (Boublik *et al.*, 1989a,b; Wahlestedt *et al.*, 1990; Shen *et al.*, 1991), and these responses are also abolished by pretreatment with histamine H_1-receptor antagonists (Shen *et al.*, 1991). The NPY-evoked histamine release from mast cells seems not to be mediated by Y_1/Y_2 receptors (Wahlestedt *et al.*, 1990a; Grundemar & Håkanson, 1990). In the present study we sought to determine which NPY analogues are capable of activating the vascular pressor receptors for NPY. It was shown in the mepyramine-pretreated rat that NPY 1–36 and the Y_1 receptor-selective analogue, [Pro^{34}]-NPY, were equally effective in increasing AP, whereas NPY 2–36 was much less potent and NPY 4–36 and NPY 11–36 were inactive. The present results suggest that the hypertensive response to NPY reflects activation of postjunctional Y_1 receptors. The low efficacy of the C-terminal NPY fragments on the AP may in addition reflect a more rapid metabolism compared to the parent molecule. The lack of reflex bradycardia in response to the NPY-evoked pressor effects may be due to the low HR (approx. 250 b.p.m.) in ketamine-xylazine anaesthetized rats compared to conscious or pentobarbitone anaesthetized rats where the HR is about 350–400 b.p.m.

NPY constricts coronary vessels of several species (Allen *et al.*, 1983; Rioux *et al.*, 1986; Macho *et al.*, 1989). In the perfused rat coronary vessels, NPY 1–36, [Pro^{34}]-NPY, NPY 2–36 and NPY 5–36 concentration-dependently increased the coronary resistance. NPY 1–36 and [Pro^{34}]-NPY were roughly equipotent whereas NPY 2–36 and NPY 5–36 were about 7 and 20 times less potent than NPY 1–36, respectively. NPY 11–36, NPY 20–36, NPY 22–36 had a slight effect only at very high concentrations; NPY 23–36 was inactive. The rapid loss of potency induced by progressive N-terminal truncation is in agreement with previous results on guinea-pig coronary vessels (Rioux *et al.*, 1986). Thus, the vasoconstriction evoked by NPY in the rat coronary vessel seems to be mediated predominantly by Y_1 receptors.

In the guinea-pig isolated inferior caval vein [Pro^{34}]-NPY, NPY 1–36, and NPY 2–36 were very effective in inducing a concentration-dependent contraction. [Pro^{34}]-NPY was more potent than NPY 1–36 while NPY 2–36 was equipotent with NPY 1–36. NPY 4–36, NPY 5–36 and NPY 11–36 were much less potent than the parent molecule. Nonetheless, in comparison with the perfused coronary vessels, several C-terminal fragments (longer than 11–36) were relatively more effective in this preparation, suggesting the presence of both Y_1 and Y_2 receptors in the vein.

The curves illustrating the displacement of [^{125}I]-PYY from rat aortic smooth muscle cells by the NPY-related peptides are consistent with a homogeneous population of Y_1 receptors, since [Pro^{34}]-NPY displayed the same affinity as NPY 1–36 on the binding sites and NPY 13–36 had 2–3 orders of magnitude lower affinity. The corresponding displacement curves for the smooth muscle cells from the rat caval vein suggest a mixed population of Y_1/Y_2 receptors since both [Pro^{34}]-NPY and NPY 13–36 displayed a high affinity for the binding sites. For detailed studies of which parts of the ligand are involved in binding, Y_1 and Y_2 receptors can be studied selectively in neuroblastoma cell lines that seem to express either of the two receptor subtypes (Schwartz *et al.*, 1990; Wahlestedt *et al.*, 1990a).

In conclusion, this study has shown the presence of Y_1 receptors in both arteries and veins. The NPY-evoked pressor response in the whole rat and in the coronary vessels seems to reflect the activation of Y_1 receptors, and the characteristics of NPY binding to aortic smooth muscle cells correspond to such receptors. In the caval vein, the contractile effects and the binding characteristics of the NPY-related peptides suggest a mixed population of Y_1 and Y_2 receptors.

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