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Evidence for different pre- and post-junctional receptors for neuropeptide Y and related peptides

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Summary

The effects of neuropeptide Y (NPY), peptide YY (PYY), desamido-NPY and five C-terminal fragments of NPY or PYY were tested on different smooth muscle preparations in vitro. The fragments were NPY 19–36, NPY 24–36, PYY 13–36, PYY 24–36 and PYY 27–36. NPY and PYY appear to exert three principally different effects at the level of the sympathetic neuroeffector junction. Firstly, they have a direct post-junctional effect, leading to constriction of certain blood vessels; this was studied on the guinea-pig iliac vein. Secondly, they potentiate the response to various vasoconstrictors; this was studied on the rabbit femoral artery and vein, using noradrenaline and histamine, respectively, as agonists. Thirdly, NPY and PYY act prejunctionally in that they suppress the release of noradrenaline from sympathetic nerve endings upon stimulation; this was studied in the rat vas deferens.

NPY and PYY were approximately equipotent in constricting the guinea-pig iliac vein, while desamido-NPY and the fragments were without effect. Desamido-NPY and the fragments were ineffective also in potentiating the response to noradrenaline in the rabbit femoral artery, nor did they potentiate the response to histamine in the rabbit femoral vein. NPY and PYY potentiated the response to noradrenaline in the artery, as well as the response to histamine in the vein. The NPY- and PYY-induced suppression of noradrenaline release from the prostatic portion of the rat vas deferens was reproduced by PYY 13–36 but not by the shorter fragments nor by desamido-NPY.

In conclusion, a C-terminal portion seems to be sufficient for exerting the prejunctional effect of NPY and PYY, while the whole sequence seems to be required

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for post-junctional (direct and modulatory) effects. An amidated C-terminal is crucial for maintaining the biological activity of NPY. Desamido-NPY and the fragments that were inactive as agonists also seemed inactive as antagonists.

neuropeptide Y; peptide YY; neurotransmission; neuromodulation; sympathetic; blood vessels; vas deferens; receptors

Introduction

Neuropeptide Y (NPY) and peptide YY (PYY) are 36-amino acid peptides belonging to the pancreatic polypeptide (PP) family. They were recently isolated from the porcine brain [1] and gut [2], respectively. NPY has been shown by immunocytochemistry to have a wide distribution, being present in a variety of central [see, e.g., 3] and peripheral neurones [cf. 4]. PYY, on the other hand, occurs predominantly in endocrine cells in the gut [5], but it is also present in the brain [6].

Interestingly, in several locations NPY coexists with catecholamines. NPY occurs in noradrenaline (NA) and adrenaline neurones in the brain stem [7] and in sympathetic neurones [4,8].

NPY has been attributed with a variety of functional roles. A vasoconstrictor effect has been demonstrated in vivo [8] and in vitro [9,10]. Besides the direct vasoconstrictor effect, NPY potentiates NA-evoked vasoconstriction post-junctionally [10–12]. Pre-junctionally, NPY has been shown to inhibit the release of NA [13–16]. In addition to these three actions of NPY (direct, and pre- and post-junctional modulatory effects) which have been observed in the periphery, NPY has been claimed to upregulate α_2-adrenoceptors in the rat brain stem [17].

The aim of this study was to compare the effects of a series of NPY and PYY fragments with the effects of the full peptides. We decided to study the guinea pig iliac vein (direct effect), the rabbit femoral artery and vein (post-junctional modulatory effect), and the rat vas deferens (pre-junctional modulatory effect).

Materials and Methods

Blood vessels

Adult male and female guinea-pigs and rabbits were killed by a blow on the neck and exsanguinated. The guinea-pig iliac vein (GPIV) (close to the cava) and the rabbit femoral artery (RFA) and vein (RFV) were rapidly taken out and placed in ice-cold Krebs solution (for composition see below). Care was taken to avoid stretching or other types of injuries to the vessels. Segments, 2–3 mm long, were mounted on two L-shaped metal holders (0.2 mm in diameter), one of which was connected to a force displacement transducer for continuous recording of the isometric tension on a Grass polygraph. The position of the other holder could be adjusted by means of a movable unit allowing precise predetermination of the tension [18].

The blood vessels were immersed in temperature controlled (37°C) tissue baths containing 3 or 1.5 ml of a modified Krebs solution of the following composition (mM): NaCl 133, $NaHCO_3$, 16.3, KCl 4.7, $MgCl_2$ 1.0, NaH_2PO_4 1.4, $CaCl_2$ 2.5 and glucose 7.8. The solution was aerated with 7% CO_2 in O_2 giving a pH of 7.2–7.3. The artery was given an initial tension of 5 mN and the veins 2.5 mN. This resulted in spontaneous relaxations and the position of the movable holder had to be adjusted in order to maintain tensions of 4 mN and 2 mN, respectively. After 90 min the contractile capacity of the vessels was examined by exposure to a solution containing 137 mM KCl (NaCl replaced by KCl). These contractions served as internal standards and were set as 100%. Concentration–response curves in the GPIV were obtained by applying the peptides (see Table I) in single concentrations (one concentration per specimen); from these curves pD_2 values were calculated (see below).

The effect of the peptides (Table I) on the RFA and RFV was tested in a different way: concentration–response curves for NA (RFA) and histamine (HI) (RFV) were obtained by cumulative addition. The various peptides were applied 2 min before the first application of NA or HI. The experiments were then completed within 10–12 min. Matched vascular segments were used as controls; they received the vasoconstrictor without prior exposure to the peptides. The log concentration–response relationship was approximated by linear regression of the data in the 10–90% response interval. The difference between the pD_2 values (i.e. the negative logarithm of the EC_{50} value) for NA and for HI in the absence and presence of peptide was tested for statistical significance using Student's *t*-test.

TABLE I

Amino acid sequences of porcine NPY, PYY and fragments thereof

	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
NPY 1-36	Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-Asp-Leu-Ala
Desamido-NPY 1-36	Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-Asp-Leu-Ala
PYY 1-36	Tyr-Pro-Ala-Lys-Pro-Glu-Ala-Pro-Gly-Glu-Asp-Ala-Ser-Pro-Glu-Glu-Leu-Ser
PYY 13-36	Ser-Pro-Glu-Glu-Leu-Ser

	19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
NPY 1-36	Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH_2
Desamido-NPY 1-36	Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asp-Leu-Ile-Thr-Arg-Gln-Arg-Tyr
PYY 1-36	Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2
PYY 13-36	Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2
NPY 19-36	Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH_2
NPY 24-36	Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH_2
PYY 24-36	Leu-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2
PYY 27-36	Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2

Amino acid sequences of NPY, PYY and the fragments employed in the study. Abbreviations: NPY = neuropeptide Y; PYY = peptide YY.

Vas deferens

Adult male rats were killed by cervical dislocation and exsanguinated. The prostatic and epididymal segments of the vas deferens (RVD) were dissected separately and bilaterally and placed in Krebs solution (for composition see above). 1-cm long segments were mounted vertically on Perspex holders in a 7-ml tissue bath maintained at 33°C. The mechanical activity was recorded isometrically using a Grass FT03 force displacement transducer and a Grass model 7 polygraph. Before starting the experiments the preparations were allowed to equilibrate for about 60 min with a tension of about 10 mN. Electrical field stimulation with square wave pulses (14–17 V over the electrodes, pulse duration 1 ms, frequency 0.15 Hz) was then applied by means of a pair of platinum electrodes connected to a Grass S4C stimulator. As soon as every pulse gave an identical response, a peptide (Table I) was added to the bath. The inhibition of the twitch was calculated for each concentration of the peptides and expressed in percent.

Drugs

Drugs used were neuropeptide Y (NPY, Peninsula, Belmont, CA, U.S.A.), peptide YY (PYY, Peninsula), desamido-NPY (NPY free acid) (Peninsula), (−)-noradrenaline HCl (NA, Sigma, St. Louis, MO, U.S.A.), histamine $(HCl)_2$ (HI, Sigma) and prostaglandin $F_{2\alpha}$ (Amoglandin®, Astra, Södertälje, Sweden). The following fragments of NPY and PYY were synthesized by solid-phase synthesis: NPY 19–36, NPY 24–36, PYY 13–36, PYY 24–36, and PYY 27–36. Their purity was confirmed by high-performance liquid chromatography and amino acid sequencing.

Results

Vasoconstriction (direct post-junctional effect)

Guinea-pig iliac vein (GPIV). The GPIV proved to be quite sensitive to NPY and PYY. Of many different blood vessels from a number of species it was the only peripheral one in which the peptides evoked a maximum contractile response exceeding 50% of that evoked by high K^+ (Wahlestedt and Håkanson, unpublished observations). NPY and PYY were equipotent (Fig. 1), the pD_2 values being 7.47 and 7.56, respectively. In contrast, neither desamido-NPY nor the C-terminal fragments (see Table I) produced vasoconstriction in the concentrations tested ($\leq 10^{-6}$ M). The possibility that desamido-NPY and the fragments might act as vasodilators was ruled out in experiments in which the GPIV had been precontracted with prostaglandin $F_{2\alpha}$ (10^{-6} M) (not shown). Moreover, neither desamido-NPY not PYY 13–36 (at 10^{-6} M concentration) inhibited the effect of subsequently added NPY (10^{-9}–10^{-6} M) (not shown).

Potentiation of vasoconstriction (post-junctional modulation)

Rabbit femoral artery (RFA). Neither NPY nor PYY ($\leq 10^{-6}$ M) affected the vascular tone per se. However, both peptides (3×10^{-7} M) caused a left-shift of the NA concentration–response curve (Fig. 2A, Table II). NPY was somewhat more

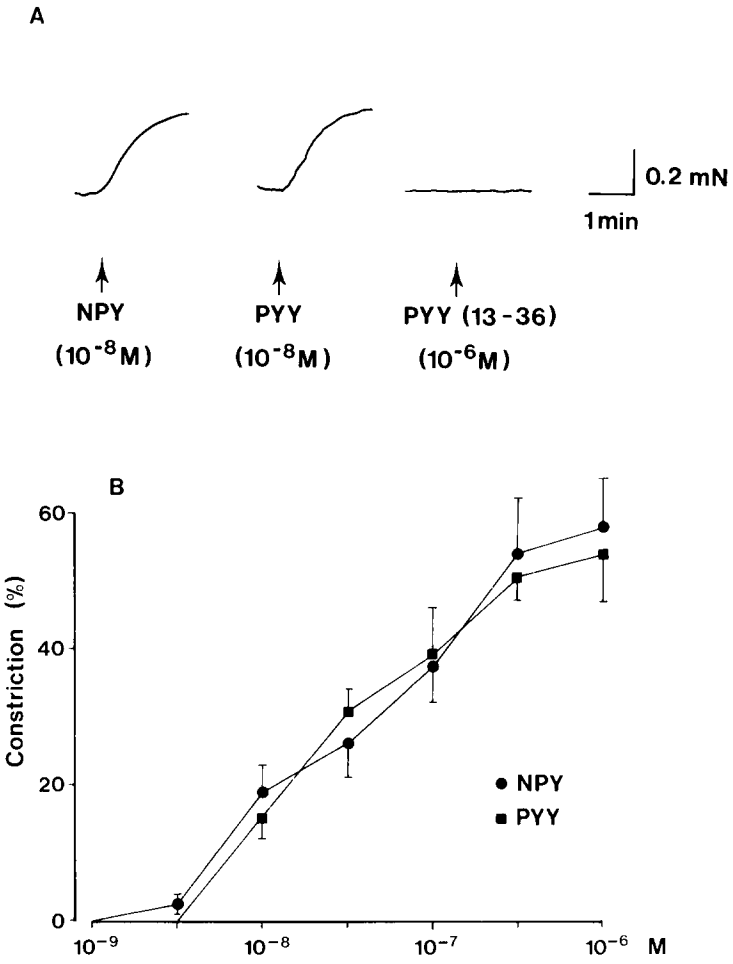

Fig. 1. Motor responses of the guinea-pig iliac vein. (A) Typical tracings following application of NPY (10^{-8} M), PYY (10^{-8} M) or PYY 13–36 (10^{-6} M). (B) Concentration–response curves illustrating the effect of NPY (●) and PYY (■). The vasoconstriction is expressed as percentage of the response to high K^+ concentration. Means ± S.E.M.

potent than PYY, the pD_2 values for the enhancement of the contractile response to 10^{-6} M NA being 8.82 and 8.58, respectively (not shown). Desamido-NPY and the fragments (Table I) did not share the potentiating ability of NPY and PYY and did not affect the vascular tone at the concentration tested (3×10^{-7} M) (Table II). Also, desamido-NPY and PYY 13–36 (10^{-6} M) did not inhibit the effect of subsequently added NPY (3×10^{-7} M) (not shown).

Rabbit femoral vein (RFV). Neither NPY, PYY, desamido-NPY nor the fragments constricted the RFV in a concentration of 3×10^{-7} M. NPY and PYY, but not desamido-NPY and the fragments, sensitized the RFV to HI, thus causing a left-shift of the concentration–response curve (Fig. 2B, Table II).

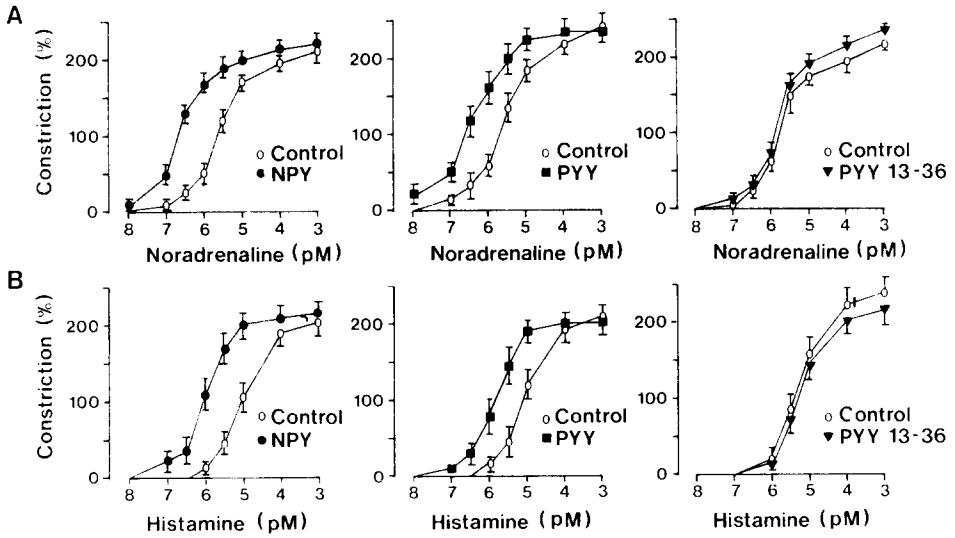

Fig. 2. (A) Noradrenaline concentration–response curves in the rabbit femoral artery in the absence or presence of NPY (3×10^{-7} M; left; ●). PYY (3×10^{-7} M; middle; ■) or PYY 13–36 (3×10^{-7} M; right; ▼). (B) Histamine concentration–response curves in the rabbit femoral vein in the absence or presence of NPY (3×10^{-7} M; left), PYY (3×10^{-7} M; middle) or PYY 13–36 (3×10^{-7} M; right). The vasoconstriction is expressed as percentage of the response to high K^+ concentration. pM stands for the negative log molar concentration. Means ± S.E.M. See also Table II.

Suppression of NA release (pre-junctional modulation)

Rat vas deferens (RVD). In the prostatic (and less so in the epididymal) segment of the RVD, NPY and PYY induced a concentration-dependent suppression of electrically evoked twitches (Fig. 3). Both peptides produced the same maximum inhibition, but NPY was slightly less potent than PYY, the pD_2 values being 7.82 and 8.16, respectively. Interestingly, one of the C-terminal fragments, PYY 13–36, was almost as effective as NPY and PYY (Fig. 3); the pD_2 value for PYY 13–36 was 7.53. The shorter fragments and desamido-NPY ($\leq 3 \times 10^{-7}$ M) were without effect. Finally, NPY 19–36 and desamido-NPY (3×10^{-7} M) did not inhibit the effect of NPY (3×10^{-10} to 3×10^{-7} M) (not shown).

The results on the agonistic properties of NPY, PYY, PYY 13–36 and desamido-NPY are summarized in Table III.

Discussion

NPY has at least three possible actions at the sympathetic neuroeffector junction: (a) direct vasoconstrictor effect, (b) potentiation of NA- (or HI)-evoked vasoconstriction, (c) suppression of stimulated NA release. All these actions can be mimicked by the structurally related PYY. In this study we have attempted to elucidate whether certain fragments of NPY and PYY share the abilities of the full peptides.

Direct vasoconstrictor effects of NPY and PYY have been demonstrated both in

TABLE II

pD_2 values for the constrictive effect of noradrenaline on the rabbit femoral artery (upper panel) and of histamine on the rabbit femoral vein (lower panel) in the absence or presence of peptide (3×10^{-7} M)

Rabbit femoral artery	pD_2 (noradrenaline)	pD_2 (noradrenaline + peptide)	*n*	Statistical significance
NPY	5.72 ± 0.04	6.60 ± 0.06	14	$P < 0.001$
Desamido-NPY	5.51 ± 0.13	5.29 ± 0.12	6	N.S.
PYY	5.82 ± 0.07	6.49 ± 0.09	9	$P < 0.001$
NPY 19–36	5.61 ± 0.14	5.49 ± 0.10	6	N.S.
NPY 24–36	5.69 ± 0.08	5.81 ± 0.13	6	N.S.
PYY 13–36	5.45 ± 0.11	5.53 ± 0.09	8	N.S.
PYY 24–36	5.52 ± 0.09	5.64 ± 0.14	7	N.S.
PYY 27–36	5.49 ± 0.12	5.47 ± 0.08	6	N.S.
Rabbit femoral vein	**pD_2 (histamine)**	**pD_2 (histamine + peptide)**	***n***	**Statistical significance**
NPY	5.41 ± 0.04	6.09 ± 0.05	12	$P < 0.001$
Desamido-NPY	5.09 ± 0.14	5.20 ± 0.11	6	N.S.
PYY	5.48 ± 0.09	5.98 ± 0.07	8	$P < 0.001$
NPY 19–36	5.28 ± 0.14	5.41 ± 0.18	5	N.S.
NPY 24–36	5.41 ± 0.10	5.39 ± 0.14	4	N.S.
PYY 13–36	5.20 ± 0.13	5.09 ± 0.11	8	N.S.
PYY 24–36	5.16 ± 0.07	5.32 ± 0.12	5	N.S.
PYY 27–36	5.36 ± 0.20	5.06 ± 0.17	6	N.S.

Mean ± S.E.M.
N.S. = Not significant.

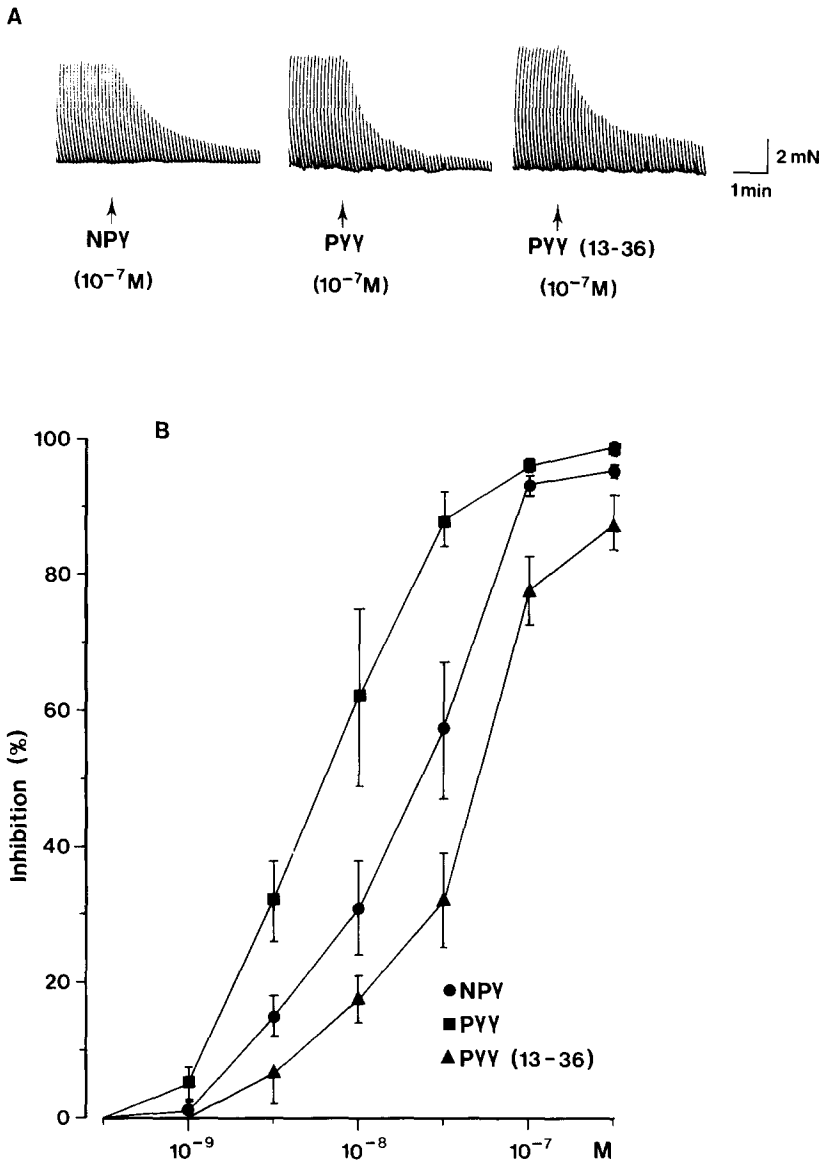

Fig. 3. Suppression by NPY, PYY and related peptides of the motor response of the rat vas deferens (prostatic part) to continuous electrical stimulation at low frequency. (A) Typical tracings following application of NPY, PYY or PYY 13–36 (10^{-7} M of either). (B) Concentration–response curves illustrating the suppressive effects of various peptides on the electrically induced twitches expressed as percentage of the twitch amplitude before application of peptide: NPY (●), PYY (■) or PYY 13–36 (▲). Each value is the mean of 4–8 observations; bars give S.E.M.

vivo [8,9,19] and in vitro [9,10]. Briefly, certain cerebral vessels and certain peripheral veins respond readily to the two peptides, while most peripheral arteries seem unresponsive ([4]; Wahlestedt and Håkanson, unpublished observations). The vasoconstrictive effect is sensitive to blockade of Ca^{2+} influx [9]. NPY and PYY had almost

TABLE III

Effects of NPY, PYY, PYY 13–36 and desamido-NPY at the sympathetic neuroeffector junction

	Direct post-junctional effect (vasoconstriction): Guinea-pig iliac vein	Post-junctional modulatory effect (potentiated NA and HI response)		Pre-junctional modulatory effect (suppressed NA release) Rat vas deferens
		Rabbit femoral artery	Rabbit femoral vein	
NPY	+	+	+	+
PYY	+	+	+	+
PYY 13–36	0	0	0	+
Desamido-NPY	0	0	0	0

All other peptide fragments tested were without effect. + indicates effectiveness; 0, indicates lack of effect.

identical effects (as studied on the guinea-pig iliac vein), while none of the fragments produced vasoconstriction.

The co-existence of NPY and catecholamines in sympathetic postganglionic neurones [cf. 4], suggests the co-operation of NPY and NA at the sympathetic neuroeffector junction. Indeed, NPY has been found to act both pre- and post-junctionally. In the rat vas deferens it was shown that NPY suppresses the release of NA [14; see also 13]; such an effect has been noted also in the rat femoral artery [15], in the guinea-pig isolated atrium [20], and in the papillary muscle from the guinea-pig right ventricle [16]. At a post-junctional site, i.e. the vascular smooth muscle, NPY was shown to greatly enhance the sensitivity to NA and other vasoconstrictors [10,11,21]. Interestingly, NA responses were potentiated in arteries only, while the HI concentration–response curve was shifted to the left in both arteries and veins [21]. In the rabbit femoral artery the potentiating action of NPY seemed to be fairly long-lasting, requiring an influx of Na^+ and the mobilization of intracellular sequestered Ca^{2+} [11]. Interestingly, the potentiation of the response to NA was found to require lower concentration of NPY than the direct vasoconstrictor effect [20]. This was the case also in the pithed rat, in which the α-adrenoceptor-stimulated blood pressure increase was enhanced by NPY in doses lower than the doses required for a direct pressor effect of NPY [12]. The combination of pre- and post-junctional effects of NPY may be physiologically desirable, leading to a rapid turn-off of transmitter release and a more pronounced effector response, respectively. From the pD_2 values of the various NPY-evoked effects at the sympathetic neuroeffector junction the following rank-order of physiological significance can be suggested: (1) post-junctional modulation of NA response (pD_2 8.82), (2) pre-junctional suppression of NA release (pD_2 8.16), and (3) direct vasoconstriction (pD_2 7.47).

In the present study, post-junctional modulatory effects of NPY, PYY, desamido-NPY and C-terminal peptide fragments were examined in two vascular preparations. The rabbit femoral artery was studied with NA as the agonist and the femoral vein with HI as the agonist. Since NPY and PYY, but not desamido-NPY and the fragments, sensitized the rabbit femoral artery to NA (and the femoral vein to HI), one may speculate that the entire sequence is required for this effect.

In contrast to the lack of effect of desamido-NPY and the peptide fragments on the blood vessel preparations, there was a marked NPY- or PYY-like effect of PYY 13–36 on the rat vas deferens, suggesting that pre-junctional receptors differ from post-junctional receptors.

In conclusion, PYY 13–36 was as effective as NPY and PYY in suppressing NA release from the rat vas deferens (pre-junctional effect). PYY 13–36 did not exert any post-junctional effects, neither direct vasoconstriction in the guinea-pig iliac vein, nor modulation of the NA and HI evoked contractions of the rabbit femoral artery and vein, respectively. Desamido-NPY and four C-terminal fragments of NPY or PYY shorter than PYY 13–36 were ineffective as agonists and antagonists in all the assay systems. Possibly, pre- and post-junctional receptors for NPY/PYY differ. The study is a first step in trying to characterize different subclasses of receptors for NPY and related peptides.

Acknowledgment

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