Effects of various neuropeptide Y/peptide YY fragments on electrically-evoked contractions of the rat vas deferens

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1 The effects of various neuropeptide Y (NPY) and peptide YY (PYY) fragments on electrically-evoked twitches in the rat isolated vas deferens were studied and compared with the effects of full length NPY and PYY. The aim was to identify the shortest NPY/PYY fragments that are capable of suppressing the contractions.

2 NPY (1–36) and C-terminal fragments of NPY (from 11–36 to 22–36) suppressed the electrically-evoked twitches in a concentration-dependent manner. On the whole there seemed to be a gradual lowering of the pIC_{50} values with progressive shortening of the NPY fragments (except for fragments 16–36 and 22–36 that had rather high pIC_{50} values). NPY 23–36, 24–36 and 25–36 suppressed the twitches at high concentrations (3 μ M). NPY 26–36 was without effect as were C-terminal carboxy-deaminated NPY and glycine extended NPY (NPY-Gly-Lys-Arg).

3 PYY (1–36) and C-terminal fragments of PYY (from 11–36 to 23–36) suppressed the electrically-evoked twitches in a concentration-dependent manner. PYY 1–36 was more potent than any of the fragments. There was a tendency for shorter fragments to have lower pIC_{50} values. PYY 24–36 and 25–36 suppressed the twitches at high concentrations (3 μ M). PYY 26–36 was without effect.

4 The findings suggest that the 12 C-terminal amino acid residues of NPY and PYY are the minimum length required to activate the Y_2 -receptor.

Introduction

Neuropeptide Y (NPY) and peptide YY (PYY) are 36-amino acid peptides that belong to the pancreatic polypeptide family (Tatemoto *et al.*, 1982). NPY is widely distributed in the peripheral nervous system and is co-localized with noradrenaline (NA) in many sympathetic fibres (Lundberg *et al.*, 1982b), notably around blood vessels (Uddman *et al.*, 1985). PYY in contrast occurs mainly in endocrine cells in the gut (Lundberg *et al.*, 1982a; Böttcher *et al.*, 1984).

NPY has been attributed a variety of functional roles (for a review see Edvinsson *et al.*, 1987). It is known to cause vasoconstriction and to enhance vasoconstriction induced by exogenous and endogenous NA. These effects are mediated via receptors (tentatively referred to as Y_1 -receptors) that require the whole NPY/PYY 36-amino acid molecule (Wahlestedt *et al.*, 1986). PYY and NPY are on the whole equipotent, whereas NPY 2–36 appears to be markedly less potent (Rioux *et al.*, 1986). Moreover, NPY can inhibit the electrically-stimulated release of NA from sympathetic fibres in the vas deferens (Lundberg & Stjärne, 1984) and in certain blood vessels (Dahlöf *et al.*, 1985). This effect is mediated via Y_2 -receptors which recognize not only full length NPY/PYY but also C-terminal fragments of NPY and PYY (Wahlestedt *et al.*, 1986).

The aim of the present study was to identify the shortest NPY/PYY fragments that can suppress electrically-evoked twitches in the rat vas deferens.

Methods

Male Sprague-Dawley rats (200–250 g) were killed by a blow on the neck and exsanguinated. The prostatic segment of each vas deferens was cut out and placed in 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. Segments 1.5 cm long were mounted vertically on Perspex holders in a 7 ml tissue bath maintained at 33°C. The mechanical activity was recorded isometrically by a Grass FT03

force displacement transducer and a Grass model 7 polygraph. Before the start of each experiment the preparations were allowed to equilibrate for 60 min with a tension of about 10 mN. Electrical field stimulation with square wave pulses (25 V, 1 ms and 0.15 Hz) was applied through a pair of platinum electrodes connected to a Grass S4C stimulator. As soon as a series of pulses gave identical responses, a peptide was added in a cumulative manner. Concentration-response curves were constructed in the concentration range 1 nM–3 μ M. The resulting inhibition of the twitch was calculated for each concentration of peptide and expressed as a percentage. The pIC_{50} values (the negative logarithm of the concentration that leaves 50% of the twitch) were estimated by linear regression analysis of the results in the 10–90% response interval. Statistical analyses of the pIC_{50} values were performed by means of unpaired Student's *t* test. The results are expressed as mean values $\pm$ s.e.mean; *n* = number of experiments.

Drugs

NPY 1–36, 16–36, 20–36, 22–36, 26–36, desamido-NPY (NPY free acid) and PYY 1–36 were purchased from Peninsula, UK. NPY 23–36, 24–36, 25–36, and PYY 23–36, 24–36, 25–36, 26–36 were synthesized by solid-phase synthesis and purified to at least 96% by h.p.l.c. (Dr H. Franzén, Dept. of Medical Chemistry, University of Lund, Sweden). NPY 11–36, 12–36, 13–36, 14–36, 15–36; PYY 11–36, 12–36, 13–36, 14–36 and 15–36 were synthesized by solid-phase synthesis and purified to at least 96% by h.p.l.c. (W. Krzeminsky, Ferring AB, Malmö, Sweden). NPY 17–36 was a gift from Dr N. Yanaihara, Shizouka, Japan. NPY-Gly-Lys-Arg was a gift from Dr. T. Bartfai, Stockholm, Sweden. All peptides were dissolved in and diluted with 0.9% saline.

Results

NPY 1–36 and truncated NPY fragments from 11–36 to 25–36 suppressed the electrically-evoked twitches of the vas deferens; NPY 26–36 was inactive (Figure 1). With NPY 1–36 and fragments longer than 23–36 the effect was concentration-dependent (Figure 2; Table 1). NPY 1–36 was more potent than all fragments except NPY 16–36, which displayed similar

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Figure 1 Tracings showing the effects of (a) neuropeptide Y (NPY) 1-36, (b) peptide YY (PYY) 1-36, (c) NPY 25-36, (d) PYY 25-36, (e) NPY 26-36 and (f) PYY 26-36 on electrically-evoked twitches in the isolated vas deferens of the rat.

potency (Table 1). However, on the whole, there seemed to be a gradual lowering of the pIC_{50} values with progressive shortening of the NPY fragments (except for fragments 16-36 and 22-36 that had unexpectedly high pIC_{50} values). High concentrations ($3 \mu M$) of NPY 23-36, 24-36 and 25-36 suppressed the

Figure 2 Concentration-response curves illustrating the suppression of electrically-evoked twitches of rat vas deferens in response to increasing concentrations of neuropeptide Y (NPY) 1-36 (●), peptide YY (PYY) 1-36 (○) (a) and of NPY 15-36 (■), PYY 15-36 (□) (b). Each point represents the mean and vertical lines indicate s.e.mean.

twitches by $11.1 \pm 3.38\%$ ($n = 8$), $23.4 \pm 5.44\%$ ($n = 6$) and $12.2 \pm 4.75\%$ ($n = 4$), respectively. In contrast, desamido-NPY ($n = 4$) and the glycine-extended 39 amino acid peptide NPY-Gly-Lys-Arg ($n = 6$) failed to reduce the twitches even at high concentrations ($3 \mu M$). PYY 1-36 and truncated fragments of PYY from 11-36 to 25-36 suppressed the electrically-evoked twitches of the vas deferens; PYY 26-36 was inactive (Figure 1). With PYY 1-36 and fragments longer than 24-36 the effect was concentration-dependent (Figure 2; Table 1). PYY 1-36 was somewhat more potent than NPY 1-36, $P < 0.01$. There was a tendency for the shorter PYY fragments to have progressively lower pIC_{50} values, and PYY 1-36 was more potent than any of these fragments (Table 1). PYY 24-36 and PYY 25-36 ($3 \mu M$) suppressed the twitches by $19.2 \pm 3.63\%$ ($n = 6$) and $32.1 \pm 3.20\%$ ($n = 4$), respectively.

Discussion

NPY and PYY display significant sequence homology (Table 2). In this study, both NPY 1-36 and NPY fragments (from 11-36 to 22-36) suppressed the electrically-evoked twitches in the vas deferens in a concentration-dependent manner. This was also the case for PYY 1-36 and the PYY fragments from 11-36 to 15-36 as well as PYY 23-36. NPY 1-36 and PYY 1-36 were more potent than their respective fragments, except NPY 16-36 which displayed a potency similar to that of NPY 1-36. NPY 23-36, 24-36, 25-36 and PYY 24-36, 25-36 suppressed the twitches at high concentrations, but neither NPY 26-36 nor PYY 26-36 had any effect. This indicates that the 12 C-terminal amino acids represent the minimum length required for activating the Y_2 -receptor. Interestingly, PYY 23-36 was more potent than NPY 23-36. The N-terminus of PYY 23-36 is serine. NPY 22-36 which was almost as effective as PYY 23-36 also starts with serine, suggesting that serine in this position may play an important role. The residues 15-31 of NPY and PYY contain a basic hydrophilic sequence, the so called PP-fold, and the C-terminal 32-36 pentapeptide sequence forms a hydrophobic tail (Glover *et al.*, 1985). Both these parts are highly conserved among the

Table 1 Ability of neuropeptide Y (NPY) and peptide YY (PYY) and C-terminal fragments of NPY/PYY to suppress the electrically-evoked twitches of rat vas deferens

Peptide	Inhibitory effect	pIC_{50}	n	P
NPY 1-36	+	7.77 ± 0.09	4	—
NPY 11-36	+	7.15 ± 0.47	4	<0.05
NPY 12-36	+	6.35 ± 0.30	4	<0.001
NPY 13-36	+	6.79 ± 0.24	4	<0.001
NPY 14-36	+	6.43 ± 0.36	8	<0.001
NPY 15-36	+	6.43 ± 0.22	4	<0.001
NPY 16-36	+	7.36 ± 0.20	4	NS
NPY 17-36	+	5.78 ± 0.05	4	<0.001
NPY 20-36	+	5.85 ± 0.14	4	<0.001
NPY 22-36	+	6.22 ± 0.14	8	<0.001
NPY 23-36	(+)		8	
NPY 24-36	(+)		6	
NPY 25-36	(+)		4	
NPY 26-36	0		4	
PYY 1-36	+	8.27 ± 0.04	4	—
PYY 11-36	+	7.32 ± 0.08	4	<0.01
PYY 12-36	+	7.06 ± 0.20	6	<0.001
PYY 13-36	+	6.35 ± 0.26	4	<0.001
PYY 14-36	+	6.58 ± 0.17	4	<0.001
PYY 15-36	+	7.14 ± 0.11	6	<0.001
PYY 23-36	+	6.48 ± 0.09	4	<0.001
PYY 24-36	(+)		6	
PYY 25-36	(+)		4	
PYY 26-36	0		4	

Data shown are means $\pm$ s.e.mean.

P, the statistical significance of the difference between pIC_{50} values for NPY 1-36 or PYY 1-36 on the one hand and for the respective fragments on the other. NS = not significant. +, inhibition 100% or close to 100% can be achieved. (+), the inhibitory effect is not close to 100% even at a concentration of $3 \mu M$ and hence pIC_{50} cannot be calculated. 0, no inhibitory effect.

Table 2 Amino acid sequences of porcine neuropeptide Y (NPY) and peptide YY (PYY)

	1	5	10	15	20	25	30	35
NPY porcine	<u>Y</u>	<u>P</u>	<u>S</u>	<u>K</u>	<u>P</u>	<u>D</u>	<u>N</u>	<u>P</u>
PYY porcine	<u>Y</u>	<u>P</u>	<u>A</u>	<u>K</u>	<u>P</u>	<u>E</u>	<u>A</u>	<u>P</u>

Identical residues are underlined.

members of the PP family (Glover *et al.*, 1985). Recently, a potent NPY agonist was synthesized by linking NPY 1–4 via epsilon-aminocaproic acid to the C-terminal peptide amide segment 25–36 (Beck *et al.*, 1989). This novel peptide excites both Y₁- and Y₂-receptors *in vivo* as well as *in vitro*.

It has been claimed that the amidated C-terminal of NPY/PYY is crucial for maintaining biological activity (Wahlestedt *et al.*, 1986). In this study, we confirmed that both desamido-NPY and NPY-Gly-Lys-Arg, the glycine-extended 39 amino acid precursor of NPY, are inactive.

In conclusion, the results of this study suggest that C-terminal fragments of NPY (from 11–36 to 22–36) and PYY (from 11–36 to 23–36) are fairly potent agonists, acting upon Y₂-receptors to suppress electrically-evoked twitches in the isolated vas deferens of the rat. Shorter fragments (NPY 23–36, 24–36, 25–36 and PYY 24–36, 25–36) are active only at very high concentrations. It appears that to be activated the Y₂-receptor requires the 12 C-terminal amino acid residues.

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