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Neuropeptide Y (NPY) and peptide YY (PYY) effects in the epididymis of the guinea-pig: evidence of a pre-junctional PYY-selective receptor

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1 The effects of peptide YY (PYY), neuropeptide Y (NPY) and structurally related peptides upon field stimulation-induced and phenylephrine-mediated contractile responses in the cauda epididymis of the guinea-pig were investigated.

2 Preparations of cauda epididymis responded to field stimulation with contractions which were completely attenuated by both the neurotoxin, tetrodotoxin (500 nM), and also by the α-adrenoceptor antagonist, phentolamine (3 μM). PYY ($n=7$) and the truncated peptide analogue PYY(3–36) ($n=5$) inhibited field stimulation-induced contractions (pIC_{50} + s.e.mean: 8.9 ± 0.2 and 9.4 ± 0.2, respectively). Pancreatic polypeptide (PP, up to 1 μM, $n=6$), NPY (up to 100 nM, $n=6$) and the NPY analogues [Leu^{31},Pro^{34}]NPY ($n=6$) and NPY (13–36) (both up to 1 μM, $n=5$) had no significant effect.

3 The NPY Y_1 receptor antagonist BIBP3226 ((**R**)-N2-(diphenylacetyl)-N[(4-hydroxyphenyl)-methyl]-argininamide) at 750 nM ($n=6$) and 7.5 μM ($n=6$) did not affect the PYY-mediated inhibition of field stimulation-induced contractions (pIC_{50} 8.9 ± 0.3 and 9.0 ± 0.3, respectively). In the presence of BIBP3226 (7.5 μM), NPY ($n=6$) inhibited field stimulation-induced contractions (pIC_{50} 8.0 ± 0.2).

4 NPY, PYY and PYY(3–36) inhibited [^{3}H]-noradrenaline release from preparations of epididymis (pIC_{50} values 7.9 ± 0.7, 9.6 ± 0.8 and 10.0 ± 0.9, respectively, all $n=6$). The agonists PP and [Leu^{31},Pro^{34}]PYY (both up to 100 nM) were without significant effect (both $n=6$).

5 In preparations of cauda epididymis, stimulated with threshold concentrations of the α_1-adrenoceptor agonist, phenylephrine (1 μM), both NPY ($n=6$) and PYY ($n=7$) elicited concentration-dependent increases in contractile force (with pEC_{50} values of 8.9 ± 0.2 and 8.6 ± 0.1, respectively). The effects of both NPY ($n=6$) and PYY ($n=6$) were antagonized by preincubation with BIBP3226 (75 nM; apparent $pK_B\pm$s.e. values 8.3 ± 1.0 and 8.2 ± 0.6, respectively). The peptide analogues NPY(13–36) ($n=5$), PYY (3–36) ($n=7$) and [Leu^{31},Pro^{34}]NPY ($n=5$) did not significantly augment responses to threshold concentrations of phenylephrine.

6 These results are consistent with the proposal that distinct NPY receptors mediate the (prejunctional) inhibition of field stimulation-induced contractions and the (postjunctional) potentiation of responses to phenylephrine in the cauda epididymis of the guinea-pig. The rank order of agonist potency (NPY ≥ PYY >> NPY(13–36), [Leu^{31},Pro^{34}]NPY and PYY(3–36) and the high potency of BIBP3226 indicate that the postjunctional receptor may be Y_1-like. The rank orders of agonist potency in inhibiting field stimulation-induced contractile responses and [^{3}H]-noradrenaline release (PYY(3–36) ≥ PYY > NPY >> PP, NPY(13–36), [Leu^{31},Pro^{34}]NPY and PYY(3–36) ≥ PYY > NPY >> PP,[Leu^{31},Pro^{34}]PYY, respectively) are consistent with the action of these peptides at a PYY-preferring receptor subtype, which may be distinct from the presently characterized NPY receptor subtypes.

Keywords: Peptide YY (PYY); neuropeptide Y (NPY); PYY receptors; NPY receptors; guinea-pig epididymis; BIBP3226

Introduction

Neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP) are members of a family of neuromodulatory peptides involved in the regulation of smooth muscle contractility and multiple central effects including behaviours such as food intake and anxiety (reviewed in Wahlestedt & Reis, 1993). These peptides interact with at least six different receptor subtypes (Y_1–Y_6); cDNAs for Y_1-, Y_2-, Y_4-, Y_5-receptors and a sixth related receptor have been cloned (Herzog *et al*., 1992; Larhammar *et al*., 1992; Bard *et al*., 1995; Gerald *et al*., 1995; 1996; Lundell *et al*., 1995; Rose *et al*., 1995; Gehlert *et al*., 1996; Gregor *et al*., 1996; Matsumoto, *et al*., 1996; Weinberg *et al*., 1996) and share homology with the superfamily of G protein-coupled receptors. NPY and PYY are approximately equipotent in assays of cultured cells expressing Y_1, Y_2 and Y_5 receptors (Gerald *et al*., 1996). In contrast the Y_3 receptor has a high affinity for NPY, but not PYY, while the Y_4 receptor has a high affinity for PP, but not for NPY nor PYY (reviewed in Gehlert, 1994; Gerald *et al*., 1996). Data have also been presented for a PYY-preferring response where PYY is 3 to 6 fold more potent than NPY (Laburthe *et al*., 1986; Voisin *et al*., 1990).

Immunohistochemical studies have shown that the innervation (from the anterior major pelvic ganglion) to the vas deferens of the guinea-pig contains NPY immunoreactive fibres (Dhami & Mitchell, 1994). In addition both PYY and NPY inhibit field stimulation-induced contractions in the vasa deferentia of rabbit, rat, mouse and guinea-pig (Lundberg & Stjärne, 1986; Ellis & Burnstock, 1990; Grundemar & Håkanson, 1990; Doods & Krause, 1991; Selbie *et al*., 1996). The receptors mediating these effects have been shown to be prejunctional Y_1-like in the rabbit and Y_2-like in the rat (Grundemar & Håkanson, 1990; Doods & Kause, 1991; Palea *et al*., 1995). In contrast to these studies of peptide receptors in the vasa deferentia, there is little evidence to indicate any functional role of neuropeptides in the cauda epididymis, a tissue which is anatomically contiguous with the vas deferens and, like the vas deferens, receives innervation from the hypogastric nerve (Mitchell, 1935). In the present study we investigated the pre- and postjunctional effects of NPY, PYY and related pep-

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tides in the cauda epididymis of the guinea-pig. A preliminary account of some of this work has been previously presented to the British Pharmacological Society (Selbie *et al*., 1996).

Methods

Animals

Duncan-Hartley guinea-pigs (600–1000 g) were killed and the vasa deferentia and testis removed. The vasa deferentia were bisected into prostatic and epididymal halves, the epididymis was unravelled and cut into sections approximately 15 mm long. All tissues were used for either contractility or [^{3}H]-noradrenaline release studies.

Field stimulation-induced contractility studies

Preparations of epididymis and prostatic vasa deferentia were placed into organ baths, containing modified Krebs solution (mM: NaCl 118, KCl 4.7, Mg_2SO_4 0.45, K_2HPO_4 2.5, $NaHCO_3$ 25, $CaCl_2$ 1.9, glucose 11) gased with O_2(95%):CO_2(5%) at 35–36°C, connected to a Grass FTO3 force-displacement transducer and suspended under 0.35 g and 1 g resting force, respectively. Recordings of contractile force were made with a Grass (model 79D) chart recorder. For measurement of field stimulation-induced contractions, tissues were allowed 40 min equilibration before field stimulation with 10 s trains of pulses (9 Hz, 0.1 ms duration; supramaximal voltage) every 14 min. Frequency-effect responses to field stimulation (0.1 ms duration, supramaximal voltage, 1 s trains; $n=6$, data not shown) showed that the mean (±s.e.mean) response to 9 Hz stimulation is 19±4% of the maximal response to field-stimulation (60 Hz). Increasing the train length (up to 30 s) increased the magnitude of the contractions but caused tissue fatigue ($n=4$, data not shown). We therefore used a train length of 10 s (9 Hz, 0.1 ms duration; supramaximal voltage) to give reproducible contractions throughout the experiment (see Figures 1 and 2c).

Peptides were added to organ baths at least 2 min before field stimulation and tissues were washed 3–5 times (bath volume) with fresh Krebs solution following each field stimulation. Only one peptide concentration-response curve was constructed on any tissue. The Y_1 receptor selective antagonist BIBP3226 (Rudolf *et al*., 1994), 750 nM or 7.5 μM, was added at least 30 min before the addition of agonists and remained in contact with the tissue throughout the duration of the experiment. The peak responses to field stimulation were recorded in the absence and presence of peptides. Responses to field stimulation in the presence of peptides are expressed as a percentage of the control (agonist-free) response (S1). At the end of some experiments the α-adrenoceptor antagonist, phentolamine (3 μM), or the neurotoxin, tetrodotoxin (500 nM), was added to the preparations of epididymis.

Effects of peptides on contractile responses to phenylephrine

Tissue contractility was determined by the addition of a near-maximal concentration of phenylephrine (PE, 30 μM; Haynes & Hill, 1996), tissues were then washed five times and left for at

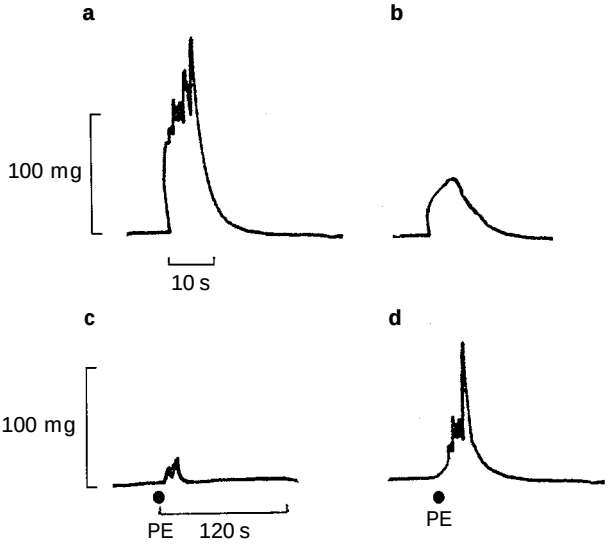

Figure 1 Effects of PYY in preparations of cauda epididymis. (a) The response to field stimulation only. (b) The response to field stimulation in the presence of PYY (100 nM). (c) The effects of a threshold concentration of phenylephrine (PE, 1 μM). (d) The effects of phenylephrine (PE, 1 μM) in the presence of PYY (100 nM). In both cases PYY was added 120 s before either field stimulation or phenylephrine addition.

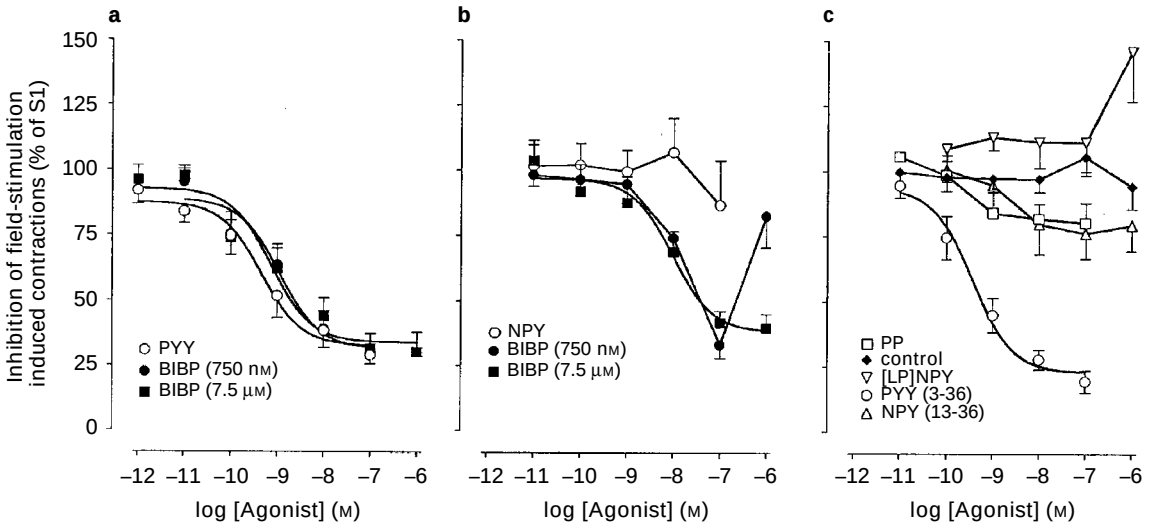

Figure 2 Inhibition of field stimulation-induced contractions in the epididymis of the guinea-pig. Peptide agonists were added to preparations 90–120 s before field stimulation. (a) The responses to PYY in the absence and presence of BIBP3226 (750 nM and 7.5 μM). (b) The responses to NPY in the absence and presence of BIBP3226 (750 nM and 7.5 μM). (c) The effects of the other peptide agonists NPY (13–36), [Leu^{31},Pro^{34}]NPY ([LP]NPY), PYY(3–36) and PP. Time control, or vehicle only, responses are also shown. The results are expressed as the percentage of the first (agonist-free) response to field stimulation. Each symbol shows the mean and vertical lines s.e.mean from 5–7 experiments. Time controls were from 11 experiments. In some cases lines and s.e.mean were omitted for clarity.

least 20 min. A threshold concentration of PE (1 μM) was added to tissues (60 s contact time), preparations were washed with fresh Krebs and left for 13 min after which PE was re-applied. Peptides or peptide vehicle (time control) were added 90–120 s before the re-addition of PE (1 μM). The responses to PE (1 μM) in the presence of peptide agonists were standardized by subtraction of the PE only response of each tissue and are shown as the net increase in (mg) force. In some experiments, tissues were incubated with the Y_1 receptor antagonist BIBP3226 (75 or 750 nM) throughout the experiment.

[^{3}H]-noradrenaline release

Preparations of epididymis were incubated, for 60 min (at 36°C), in modified Krebs solution containing [^{3}H]-noradrenaline (200 nM, 10.4 Ci mmol^{-1}, DuPont, U.K.) and ascorbic acid (100 μM) in a final volume of 4 ml. Preparations were then suspended under 0.4 g resting force in isolated tissue chambers (2 ml) and perfused at 1.5 ml min^{-1} with fresh Krebs solution (containing β-oestradiol and cocaine, both at 10 μM), at 34–35°C, and bubbled with O_2:CO_2 (95:5), for 90 min. After this equilibration period, fractions were collected each minute. For the four minutes before (the first) field-stimulation (S1) the Krebs solution was exchanged for one containing peptide vehicle, preparations were stimulated (10 s trains at 9 Hz, 0.1 ms duration and supramaximal voltage) and then washed for 20 min with fresh Krebs solution. This protocol was repeated for the subsequent stimulations (S2, S3 and S4) where preparations were superfused with agonists or vehicle. In these preparations basal tritium ([^{3}H]-noradrenaline) release was 328 ± 60 d.p.m. min^{-1} ($n=6$). Following field-stimulation, and in the absence of agonists, the maximal tritium release was 519 ± 85 d.p.m. min^{-1} ($n=6$). For each experiment the maximal tritium release is shown as the net increase over basal tritium release expressed as a percentage of basal tritium release. An identical method was employed to calculate the effects of agonists upon subsequent field-stimulations (S2, S3 and S4). In time control preparations the increase in tritium release following S1, S2, S3 and S4 was 63 ± 13, 69 ± 34, 65 ± 11 and 68 ± 18% over basal, respectively.

Statistics

Estimates of pEC_{50}, pIC_{50} and maximum response were generated by means of a four-parameter logistic curve fitting and graphics programme PRISM 2.01 (GraphPad Software Inc., San Diego). Differences between concentration–response curves were determined by the use of an iterative curve fitting programme, FLEXIFIT (see Guardabasso *et al*., 1988). Significant changes in data sets were determined with an F test, one-way ANOVA or Student's t test. $P<0.05$ is taken as the level of significance. Apparent pK_B values were determined by use of the Gaddum equation: $pK_B = \log$ [concentration-ratio -1] $-\log$ [antagonist concentration].

Drugs

Peptides (human PYY, PYY(3–36), NPY, [Leu^{31},Pro^{34}]PYY and PP, and porcine NPY(13–36) and [Leu^{31},Pro^{34}]NPY) were obtained from Calbiochem-Novabiochem Ltd (U.K.) and Peninsula, (U.S.A.). Peptides were dissolved in 0.01 M acetic acid in H_2O and stored at -20°C. On the day of use peptides were diluted with modified Krebs buffer containing 0.1% bovine serum albumin. Phenylephrine HCl and tetrodotoxin were obtained from the Sigma Chemical Co. (U.K.). Tetrodotoxin was stored as a stock solution in acidified ethanol. Phentolamine mesylate (Rogitine) was from Roche. The Y_1 receptor antagonist BIBP3226 ((**R**)-N2-(diphenylacetyl)-N-[(4-hydroxyphenyl)methyl]-argininamide) was kindly provided by Dr Karl Thomae GmbH and dissolved in 10% dimethylsulphoxide in H_2O, then diluted in 0.9% saline.

Results

Contractile responses to field stimulation

Preparations of cauda epididymis responded to electrical field-stimulation (10 s trains, 9 Hz, 0.1 ms duration, supramaximal voltage) with contractions (see Figure 1 for typical responses). Field stimulation-induced contractile responses of the epididymis changed significantly over the duration of each experiment ($P<0.05$, one-way ANOVA); the sixth stimulation was 84 ± 6% of the response to the first stimulation, see Figure 2c. The contractile responses of the epididymis were completely attenuated by phentolamine (3 μM, $n=5$, data not shown) and also by tetrodotoxin (500 nM, $n=4$, data not shown).

Effects of peptide agonists on field stimulation-induced contractions

Both PYY and NPY inhibited field stimulation-induced contractions in the prostatic half of the vas deferens of the guinea-pig (Table 1). In the epididymis of the guinea-pig both PYY and the peptide fragment PYY(3–36) inhibited field stimulation-induced contractions. NPY (up to 100 nM) and NPY

Table 1 Effects of PYY and related peptides on field stimulation-induced (FS) contractions and [^{3}H]-noradrenaline ([^{3}H]-NA) release in the cauda epididymis and prostatic vas deferens of the guinea-pig

Peptide	*Vas deferens FS contractions*	*Epididymis FS contractions*	*Epididymis [^{3}H]-NA release*
PYY	9.1 ± 0.1 (77 ± 4)	8.9 ± 0.2 (69 ± 4)	9.6 ± 0.8 (46 ± 10)
PYY + BIBP (750 nM)	ND	8.9 ± 0.3 (70 ± 1)	ND
PYY + BIBP (7.5 μM)	ND	9.0 ± 0.3 (69 ± 8)	ND
PYY (3–36)	ND	9.4 ± 0.2 (77 ± 7)	10.0 ± 0.9 (60 ± 22)
NPY	8.8 ± 0.1 (67 ± 4)	<7	7.9 ± 0.7 (40 ± 10)
NPY + BIBP (7.5 μM)	ND	8.0 ± 0.2 (60 ± 5)	ND
NPY (13–36)	7.4 ± 0.1 (49 ± 5)	<6	ND
NPY (13–36) + BIBP (7.5 μM)	ND	<6	ND
[Leu^{31},Pro^{34}]NPY	ND	<6	ND
[Leu^{31},Pro^{34}]PYY	ND	ND	<7
PP	ND	<7	<7

The effects of peptides upon field stimulation (FS)-induced contractions in preparations of cauda epididymis. These data are the mean $\pm$ s.e.mean of the pIC_{50} of peptide agonist-mediated inhibition of responses to field stimulation and [^{3}H]-noradrenaline release. The mean maximal inhibition ($\pm$s.e.mean) of responses (as a percentage of S1) is shown in parentheses. In some experiments the neuropeptide Y Y_1 receptor antagonist BIB3226 (BIBP, 750 nM or 7.5 μM) was included throughout the experiment. All data shown are the mean $\pm$ s.e.mean of 5–7 experiments. The pIC_{50} of peptides which did not elicit significant effects (up to 100 nM or 1 μM) are shown as either <7 or <6, respectively. ND not determined

(13–36) (up to 1 μM) were without effect, while [Leu^{31}, Pro^{34}]NPY (only at 1 μM) significantly increased contractions, as compared to time control ($P<0.05$, Student's t test; d.f. = 10; see Figure 1 for typical trace, Figure 2a and c and Table 1).

The Y_1 receptor-selective antagonist BIBP3226 (at 750 nM and 7.5 μM) did not affect the PYY-mediated inhibition of field stimulation-induced contractions (Figure 2a, Table 1). In the presence of BIBP3226 (750 nM) NPY (up to 100 nM) inhibited field stimulation-induced contractions in the epididymis (Figure 2b). In the presence of a greater concentration of BIBP3226 (7.5 μM) NPY inhibited field stimulation-induced contractions in a concentration-dependent manner (Figure 2b and Table 1). In the presence of BIBP3226 (750 nM) NPY (13–36) (up to 1 μM) still did not inhibit field stimulation-induced contractions in preparations of epididymis ($n=4$, data not shown).

Effects of peptide agonists on [^{3}H]-noradrenaline release

In the presence of BIBP3226, NPY effectively inhibited field stimulation-induced contractions in the cauda epididymis (Figure 2b and Table 1). To confirm the action of peptide agonists at prejunctional sites in the cauda epididymis, the effects of peptides on tritium ([^{3}H]-noradrenaline) release were determined.

Field stimulation (S1) elicited a small net increase in tritium release of 63±13% over basal NPY, PYY, PYY(3–36), PP and [Leu^{31},Pro^{34}]PYY (all up to 100 nM) reduced tritium release to 38±6, 34±6, 25±14, 59±22 and 47±16% over basal, respectively ($n=5$–6). These data are also presented as a percentage inhibition of S1 in Table 1. NPY, PYY and PYY(3–36) inhibited tritium release with pIC_{50} values of 7.9±0.7, 9.6±0.8 and 10.0±0.9, respectively ($n=5$–6, Figure 3). The mean pIC_{50} value of NPY was significantly different from those of PYY and PYY(3–36) ($P<0.05$, F test, d.f. = 1,73; see Table 2). PP and [Leu^{31},Pro^{34}]PYY (1–100 nM) had no significant effects upon tritium release ($n=5$ and 6, respectively).

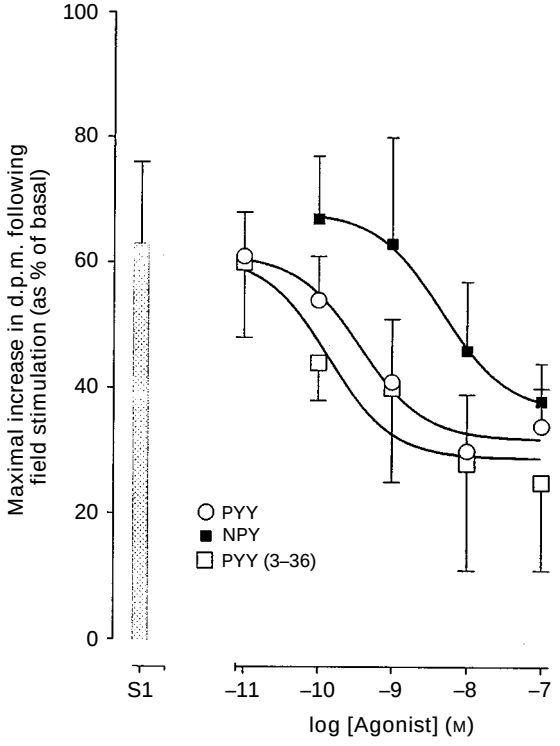

Figure 3 Effects of peptide agonists upon [^{3}H]-noradrenaline release. Preparations of epididymis were superfused with Krebs solution containing peptide agonists, NPY, PYY or PYY(3–36) for 4 min before field stimulation. The net increase in tritium release (d.p.m.) following field-stimulation is expressed as a percentage of basal tritium release. Mean±s.e.mean control responses (S1) are shown by the vertical column ($n=6$). Each symbol shows the mean and vertical lines s.e.mean from 5–6 experiments. In some cases s.e.means were omitted for clarity.

Effects of peptide agonists on contractile responses to phenylephrine

Peptide agonists (10 pM–1 μM) were added to preparations of epididymis in the presence of threshold concentration of α-adrenoceptor agonist phenylephrine (PE, 1 μM; Haynes & Hill, 1996). Both NPY and PYY significantly potentiated contractile responses to PE (1 μM) in a concentration-dependent manner (Figure 4a and b). The analogues PYY(3–36) and NPY(13–36) were ineffective, whereas the results indicate that [Leu^{31},Pro^{34}]NPY (at 1 μM) potentiates responses to phenylephrine (Figure 4c). The Y_1 receptor antagonist BIBP3226 (75 and 750 nM) shifted both NPY and PYY concentration-response curves, in parallel, to the right (Figure 4, Table 2). BIBP3226 (up to 7.5 μM) did not affect the contractile responses to threshold (1 μM) doses of PE (data not shown).

Discussion

We have shown that preparations of guinea-pig epididymis and vas deferens respond to field stimulation with contractile responses which are inhibited by peptide agonists. In contrast to preparations of vas deferens, which respond to field stimulation with purinergic (rapid twitch) and adrenergic (latent) components of contraction (Sneddon & Westfall, 1984), preparations of epididymis respond to field stimulation with contractions which are adrenergic in origin, since the α-adrenoceptor antagonist, phentolamine, abolished responses completely. Since all responses to field stimulation were, at the parameters used in this study, attenuated by the neurotoxin tetrodotoxin, it is likely that these contractile responses were entirely of neural origin. Analogous findings have also been obtained in preparations of epididymis of the rat (Ventura & Pennefather, 1992).

NPY and PYY are equipotent inhibitors of field stimulation-induced contractions in the prostatic half of the vas deferens of the guinea-pig (Table 1 and Selbie *et al*, 1996). Similar effects of NPY have been previously demonstrated in the prostatic half of the vas deferens of rabbit, rat, mouse and guinea-pig (Ellis & Burnstock, 1990; Grundemar & Håkanson, 1990; Doods & Krause, 1991) where NPY inhibited both purinergic (rapid twitch) and adrenergic (latent) components (Ellis & Burnstock, 1990). The NPY receptor involved in the inhibition of field stimulation-induced contractions in the vas deferens is, in this study, Y_2-like, since NPY and PYY are equipotent and the peptide fragment NPY(13–36) (an agonist at Y_2 and Y_5 receptors) is more potent than either [Leu^{31},-Pro^{34}]NPY (an agonist at Y_1, Y_3, Y_4 and Y_5 receptors) or PP

Table 2 Effects of PYY and NPY upon contractile responses to phenylephrine (1 μM) in the cauda epididymis of the guinea-pig

Peptide	*pEC_{50}*	*Apparent pK_b*
PYY	8.6±0.1	
PYY + BIBP3226 (75 nM)	7.4±0.2	8.2±0.6
NPY	8.9±0.2	
NPY + BIBP3226 (75 nM)	7.7±0.2	8.3±1.0

Peptides were added 90–120 s before the addition of phenylephrine (1 μM). In some cases preparations were incubated with the Y_1 receptor antagonist, BIBP3226. All data shown are the mean±s.e.mean of 6 replicate experiments.

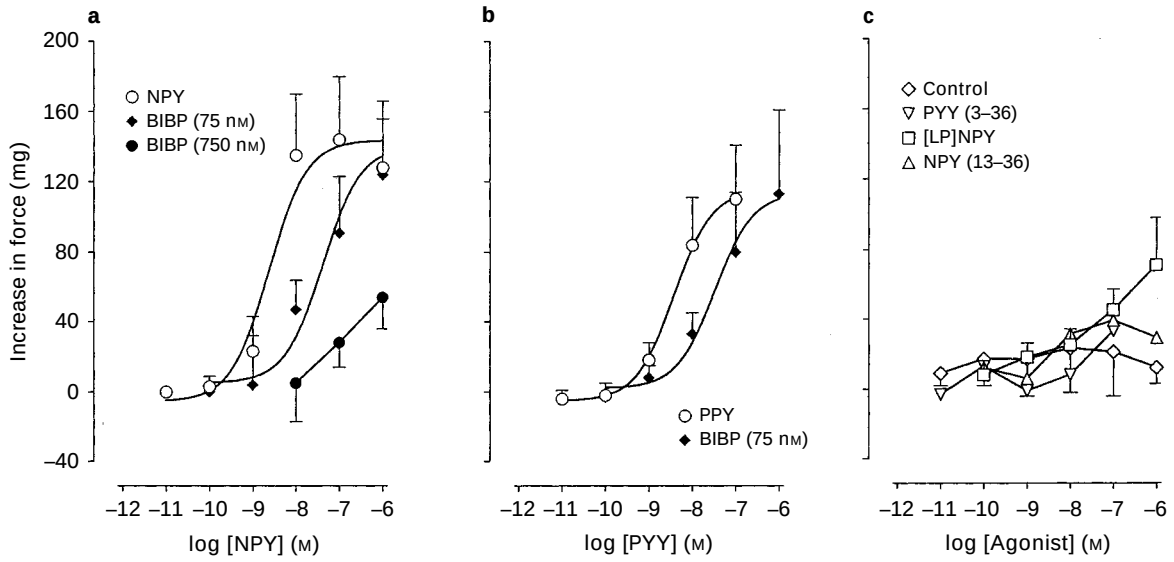

Figure 4 Effects of peptide agonists upon responses to a threshold concentration of phenylephrine (1 μM). Peptides were added 90–120 s before the addition of phenylephrine. (a) The effects of NPY in the absence and presence of BIBP3226 (75 nM and 750 nM). (b) The effects of PYY in the absence and presence of BIBP3226 (75 nM). (c) The effects of the other peptide agonists PYY(3–36), [Leu^{31},Pro^{34}]NPY ([LP]NPY) and NPY(13–36). Time control, vehicle only, tissues are also shown. The results were calculated as the net increase in (mg) force in the presence of peptide. Each symbol shows the mean and vertical line s.e.mean from 5–7 experiments. In some cases s.e.means were omitted for clarity.

(an agonist at Y_4 and Y_5 receptors) (Fuhlendorff *et al*., 1990; Wahlestedt & Reis, 1993; Dumont *et al*., 1994; Gerald *et al*., 1996; Selbie *et al*., 1996). This hypothesis is consistent with previous results describing a prejunctional Y_2 receptor in the vas deferens of the rat (Grundemar & Håkanson, 1990; Doods & Krause, 1991), but not with results describing a Y_1-like receptor in the rabbit (Palea *et al*., 1995).

In contrast to our evidence for a prejunctional, Y_2-like NPY receptor in the prostatic half of the vas deferens, we have now demonstrated that PYY and PYY(3–36), but not NPY, PP, [Leu^{31},Pro^{34}]NPY or NPY(13–36) inhibit responses to field stimulation in preparations of the cauda epididymis of the guinea-pig. This insensitivity of the field stimulation-induced contractions to NPY was surprising. Although there is evidence for a putative receptor where PYY is (up to) 6 fold more potent than NPY (Laburthe *et al*., 1986; Voisin *et al*., 1990), there are no accounts of an NPY-insensitive PYY receptor. One explanation for the lack of effect of NPY upon field stimulation-induced contractions is that NPY has postjunctional actions which mask its prejunctional actions. There is some precedent for this since, in the vas deferens of the guinea-pig, NPY both inhibits the release of ATP and noradrenaline (Ellis & Burnstock, 1990) and, also, acts postjunctionally to augment adrenoceptor and purine receptor-stimulated contractions (Stjärne *et al*., 1986; Ellis & Burnstock, 1990). To determine whether NPY and related peptides have significant postjunctional actions in the cauda epididymis, we stimulated preparations with peptide agonists (in combination with a threshold concentration of phenylephrine). In these preparations the peptide agonists elicited contractile responses with a rank order of potency of NPY $\geqslant$ PYY $>>$ [Leu^{31},Pro^{34}] NPY = NPY(13–36) = PYY (3–36). The non-peptide Y_1 receptor-selective antagonist BIBP3226 (Rudolf *et al*., 1994) blocked both the NPY- and PYY-mediated contractions of the epididymis with apparent pK_B values of approximately 8.3 and 8.2; these values are consistent with previously found pA_2 values (8.52, 8.0, 8.53 and 7.9; Abounader *et al*, 1995, Malmstrom & Lundberg, 1995 and Nilsson *et al*., 1996 and Tough & Cox, 1996, respectively), pK_i values (8.7 and 8.3; Holliday & Cox, 1996) and a pK_B value of 8.0 (Holliday & Cox, 1996). These findings lead us to conclude that the epididymis of the guinea-pig expresses a postjunctional BIBP3226-sensitive, Y_1-like, neuropeptide receptor, which potentiates α-adrenoceptor-mediated contractile responses.

Since our evidence indicates that NPY and PYY elicit contractions from preparations of epididymis, we used BIBP3226 to mask these postjunctional peptide-mediated responses. In contrast to its earlier effects, NPY, in the presence of BIBP3226 (7.5 μM), elicited a concentration-dependent inhibition of field stimulation evoked contractions. At this concentration (approximately three orders of magnitude above its apparent pK_B) BIBP3226 did not affect the PYY-mediated inhibition of responses to field stimulation. Thus the rank order of potency of agonists inhibiting the responses to field stimulation in the epididymis was PYY(3–36) $\geqslant$ PYY $>$ NPY (in the presence of BIBP3226, 7.5 μM) $>>$ [Leu^{31},Pro^{34}]NPY, NPY)13–36) and PP. Interestingly, even in the presence of BIBP3226 (750 nM) the Y_2 selective NPY agonist fragment, NPY(13–36), was still without effect upon responses to field stimulation. This latter finding indicates that the BIBP3226 insensitive, PYY-preferring response may be the result of an effect on a receptor which is distinct from the Y_2 subtype (as has been demonstrated in the rat; Doods *et al*., 1995; Jacques *et al*., 1995). This response may, therefore, be mediated through a receptor similar to that which mediates previously described PYY-preferring responses (Voisin *et al*., 1990; Sawa *et al*., 1995).

To confirm this observation of a PYY-preferring receptor inhibiting field stimulation-induced contractile responses, we measured the inhibition, by peptide agonists, of [^{3}H]-noradrenaline release in preparations of epididymis. In spite of the difficulties presented by the size of these tissues and the modest increase in tritium release following field stimulation, we obtained similar rank orders of potency of peptide agonists at inhibiting both field stimulation-induced contractions and also [^{3}H]-noradrenaline release in preparations of epididymis (PYY(3–36) $\geqslant$ PYY $>$ NPY $>>$ PP). These findings are consistent with the possibility that a prejunctional PYY-preferring receptor modulates neurotransmitter release, and therefore contractility, in the epididymis of the guinea-pig.

It is unlikely that selective enzymatic degradation of NPY can account for its lower potency (compared to that of PYY) at prejunctional receptors for the following reasons; NPY and PYY were equipotent in potentiating contractile responses to phenylephrine and, in the presence of the Y_1 receptor antagonist BIBP3226, NPY inhibited field stimulation-induced contractions. Together these data and the finding that NPY

and PYY both inhibited the release of [^{3}H]-noradrenaline are consistent with our hypothesis that both NPY and PYY act at a pre-junctional PYY-preferring receptor in the epididymis of the guinea-pig to inhibit neurotransmitter release. It is also evident that the postjunctional contractile effects of NPY mask the action of this peptide at prejunctional receptors.

In conclusion, we have shown that in the cauda epididymis, and in contrast to the vas deferens, PYY is more potent than NPY in inhibiting field stimulation-induced contractions. The finding that NPY only inhibited field stimulation induced contractile responses in the presence of BIBP3226 is consistent with the hypothesis that the action of NPY at postjunctional Y_1-like NPY receptors offsets its action at prejunctional PYY-preferring receptors. Thus our data demonstrate that neurogenic contractions are regulated by distinct pre- and postjunctional receptor subtypes. Since our evidence is not consistent with the prejunctional response being mediated by any of the Y_1-Y_5 NPY receptor subtypes, it is likely that the unique action of PYY, in the guinea-pig epididymis, is the result of activation of a distinct receptor subtype with preference for PYY. Alternatively, this receptor may represent a guinea-pig homologue of a previously cloned/characterized receptor. Confirmation of the existence of such an atypical receptor awaits the molecular cloning of the receptor cDNA. The sensitivity of contractile responses to the neuromodulatory peptides PYY and NPY may implicate one or both of these peptides in the regulation of local constrictor activities along the epididymis and vas deferens of the guinea-pig.

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