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# Failure of the putative neuropeptide Y antagonists, benextramine and PYX-2, to inhibit $Y_2$ receptors in rat isolated prostatic vas deferens

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1 The pharmacological activity of neuropeptide Y (NPY) and some analogues in inhibiting the twitch contractions induced by electrical stimulation (single pulses at 25 V, 0.15 Hz, 1 ms) in the prostatic portion of the rat isolated vas deferens was investigated. The rank order of agonist potency was: PYY > $NPY_{2-36}$ > NPY >> $NPY_{13-36}$ >> $NPY_{18-36}$ >> [$Leu^{31}$,$Pro^{34}$]NPY = hPP, which is consistent with the activation of a $Y_2$ receptor.

2 The putative $Y_1$ and $Y_2$ antagonist, benextramine (BXT), incubated at 100 $\mu$M for 10 or 60 min, was ineffective against PYY-induced inhibition of the twitch response, suggesting that the prejunctional $Y_2$ receptor in this tissue is different from the postjunctional one reported in the literature to be sensitive to BXT blockade.

3 The putative NPY antagonist, PYX-2, incubated at 1 $\mu$M for 20 min, was completely ineffective in antagonizing PYY-induced inhibition of twitches.

4 The twitch response was totally inhibited by suramin (100 $\mu$M) but was little affected by prazosin (1 $\mu$M). Futhermore, NPY was without effect on the dose-response curve to ATP in resting conditions. Taken together, these results suggest that in our paradigm, NPY inhibits the release of a purinergic neurotransmitter which mediates contraction of the prostatic portion of the rat vas deferens.

**Keywords:** Rat vas deferens; twitch contraction; NPY; [$Leu^{31}$,$Pro^{34}$]NPY; $Y_2$ receptor; $Y_1$ receptor; purinergic neurotransmission; suramin; benextramine; PYX-2; antagonism

## Introduction

Neuropeptide Y (NPY) is a 36 amino acid peptide belonging to the pancreatic polypeptide family, which has been found to be co-stored with noradrenaline (NA) and adenosine triphosphate (ATP) in noradrenergic sympathetic terminals of the mouse and human vas deferens (Stjarne *et al.*, 1986; Adrian *et al.*, 1984). It is generally accepted that NPY exerts its pharmacological effects via at least two distinct receptor subtypes, namely $Y_1$ and $Y_2$. Originally, it was proposed that $Y_1$ receptors were exclusively located postjunctionally and that they mediated direct vasoconstriction or potentiation of the NA response in several blood vessels. On the other hand $Y_2$ receptors were thought to be located exclusively prejunctionally and to be involved in the suppression of the electrically stimulated twitch contractile response of the isolated vas deferens (Wahlestedt *et al.*, 1986). However, this view has not been sustained by some recent papers e.g. using the rank order of potency exhibited by NPY and some truncated analogues, the presence of prejunctional $Y_1$ receptors in rabbit isolated vas deferens was proposed (Doods & Krause, 1991).

Many electrophysiological and pharmacological studies have been performed with the rat vas deferens in order to clarify the nature of the neurotransmitter involved in the mechanical contractile response. It was shown that responses to single stimuli consisted of two phases, a fast component resistant to prazosin and predominant in the prostatic portion and a slower one, predominant in the epididymal portion, which is blocked by $\alpha$-adrenoceptor antagonists (Brown *et al.*, 1979). Recently, it was demonstrated that the $P_2$ antagonist suramin selectively impaired the first phase of the response to single pulse field stimulation but was without effect on the second adrenergic phase, indicating the involvement of released ATP as neurotransmitter of the fast contractile response (Mallard *et al.*, 1992). In the whole isolated vas deferens of the rat a NPY-mediated depression of [$^3$H]-NA secretion evoked by trains of pulses was demonstrated (Lundberg & Stjarne, 1984). However, this peptide was ineffective in inhibiting the contractile response to single stimuli in the epididymal portion of the rat vas deferens (presumably mediated by NA release) but potently suppressed the same response evoked in the prostatic portion (Donoso *et al.*, 1988), suggesting that NPY may differentially affect NA and ATP release in epidydimal and prostatic portions of the rat vas deferens.

The aim of this study was to characterize, in the prostatic portion of the rat vas deferens, the NPY subtype involved in the suppression of twitches by use of NPY, peptide YY (PYY), human pancreatic polypeptide (hPP), the selective $Y_2$ receptor agonist $NPY_{18-36}$ (Michel *et al.*, 1990) together with the selective $Y_1$ agonist, [$Leu^{31}$,$Pro^{34}$]NPY (Fuhlendorff *et al.*, 1990). Moreover, the potential antagonistic activities of benextramine, a tetramine disulphide reported to block $Y_1$ and $Y_2$ receptors in rat isolated femoral artery (Tessel *et al.*, 1993) and of the putative NPY peptide antagonist PYX-2 (Ac-[3-(2,6-dichlorobenzyl)$Tyr^{27,36}$,D-$Thr^{32}$]NPY(27-36) amide), originally developed by Tatemoto *et al.* (1992) were tested. Furthermore, we decided to investigate whether a high dose of NPY can modify the smooth muscle response to ATP in this tissue and to test the ability of the $P_2$-purinoceptor antagonist, suramin, to suppress the twitch contractile response. A preliminary account of some of these results was presented to the British Pharmacological Society Meeting, Victoria University of Manchester, 13th-15th April, 1994 (Palea *et al.*, 1994).

## Methods

Male Sprague-Dawley rats weighing 200-300 g were killed by a blow on the neck. Bilateral prostatic segments of vasa deferentia (cut as near as possible to the prostate) were removed

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and placed in a Petri dish containing cold, oxygenated Krebs solution. After dissection of connective tissue and blood vessels, the entire prostatic segment (1.5 cm long) was mounted vertically between two platinum electrodes (separated by 3 cm) and placed in a 2 ml sylanized organ bath filled with a modified Krebs solution of the following composition (mM): NaCl 133, $NaHCO_3$ 16.3, KCl 4.7, $MgCl_2$ 1.0, $KH_2PO_4$ 1.4, $CaCl_2$ 2.0 and glucose 7.8 bubbled with 95% $O_2$ and 5% $CO_2$ at 33°C. Only two preparations were obtained from each animal and only one peptide was tested on each preparation.

The mechanical activity was recorded isometrically by a Grass FT03 force displacement transducer connected to a Linseis model 7025 polygraph. Isolated vasa deferentia were allowed to equilibrate for 90 min with a tension of 1 g applied four times during the equilibration period and washed with the fresh Krebs solution every 15 min. Electrical field stimulation was performed with single pulses (25 V, 1 ms, 0.15 Hz) and applied through a pair of platinum electrodes connected to a Grass S88 stimulator. As soon as a series of pulses gave identical responses (control contractile response) a single cumulative concentration-response curve (CRC) to each peptide was performed. The resulting inhibition of the twitch response was expressed as the percentage of the control contractile response. For each peptide the $IC_{50}$ value (that is the concentration causing a 50% inhibition of the control contractile response) was estimated by non-linear regression analysis using the ALLFIT programme (DeLean *et al.*, 1978) installed on an HP Vectra computer.

### *Effect of benextramine and PYX-2 on PYY CRC*

Benextramine tetrahydrochloride (BXT) was incubated at 100 μM for 10 or 60 min after the control contractile response to electrical stimulation (ES) was stabilized. After 10 min ES was stopped, tissues were washed and allowed to recover for 15 min, then ES was reapplied and tissues were challenged with the agonist as soon as the contractile response was stabilized. This protocol was chosen supposing BXT an irreversible antagonist of the $Y_2$ receptor, possibly, as hypothesized recently, through an interchange reaction between the disulphide bond of BXT and a receptor thiol function (Melchiorre *et al.*, 1994). Theoretically, after incubation of the tissue with an alkylating agent, it is necessary to eliminate the excess of antagonist, bound to sites other than the receptor under study, which could be released carrying out the dose-response curve to the agonist (Kenakin, 1993).

A different protocol was chosen for the peptide antagonist, PYX-2, because there is no evidence that it is an irreversible antagonist of NPY receptors. So, PYX-2 was added to the organ bath at 1 μM immediately after the contractile response had stabilized and incubated for 20 min, with no washouts, before testing the agonist.

### *Activity of suramin and NPY on ATP-induced contractions*

One serial CRC to ATP was obtained on each tissue in the absence or presence of suramin at 30, 100 and 300 μM (incubation time 60 min). Before starting the experiment, tissues were challenged with KCl 100 mM to obtain a reference contraction to which the contractile responses to ATP were normalized. Immediately after the achievement of a plateau response, tissues were washed with the fresh Krebs solution. The interval between two successive doses was 20 min and during this time, tissues were washed every 5 min. When the effect of NPY 0.3 μM was tested on ATP contractility, the peptide was added to the organ bath 2 min before ATP challenge.

### *Drugs*

All peptides were obtained from Peninsula Laboratories Europe Ltd (St. Helens, Merseyside, U.K.). Benextramine tetrahydrochloride (approximately 90% pure) and tetrodotoxin were from Sigma.

All peptides were dissolved, at a concentration of 100 μM, in twice distilled water containing bovine serum albumin (BSA) 0.5 mg $ml^{-1}$ and stored in aliquots frozen at −20°C. BXT was dissolved in distilled water.

## Results

### *Agonist activity in rat vasa deferentia*

The mean amplitude of the twitch response after stabilization (control contractile response) was 0.52±0.04 g. These responses were completely abolished by tetrodotoxin (TTX, 1 μM), so confirming their neurogenic origin.

In Figure 1 the CRC to the agonists used are shown. PYY was the most potent peptide in inhibiting the twitch response, the threshold was generally 0.1 nM. $NPY_{2-36}$ was almost equipotent to it and 5.0 times more potent than NPY. The selective $Y_1$ agonist, [Leu$^{31}$,Pro$^{34}$]NPY and hPP showed very low activity; in fact, the inhibition of the control contractile response at 1 μM (the maximal concentration that could be tested) was only 19.0±3.1% and 25.1±2.2%, respectively ($n$=6 for each). The rank order of potency was PYY > $NPY_{2-36}$ > NPY > > $NPY_{13-36}$ > > $NPY_{18-36}$ > > [Leu$^{31}$,Pro$^{34}$]NPY = hPP. In Table 1, $IC_{50}$ values (±s.e.mean) and equipotent molar ratios (e.p.m.r.) for all the peptides tested are shown.

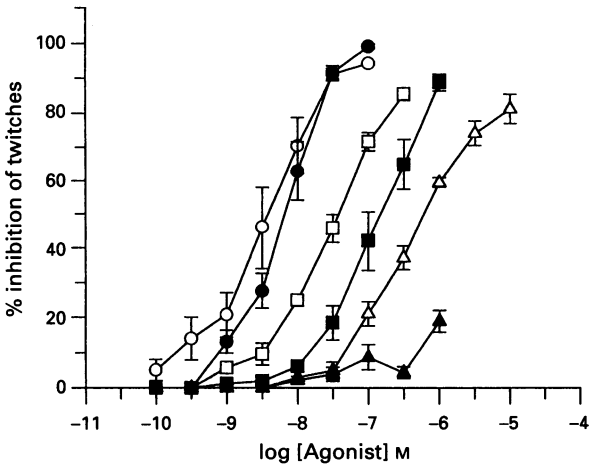


**Figure 1** Effect of NPY and related analogues on the twitch response of the prostatic portion of the rat isolated vas deferens. Data are expressed as % of the amplitude of the contractile response just before addition of the agonist to the organ bath. Each curve is the mean±s.e. of 6 experiments carried out on different tissues: PYY (○), $NPY_{2-36}$ (●) NPY (□) $NPY_{13-36}$ (■) $NPY_{18-36}$ (△) [Leu$^{31}$,Pro$^{34}$]NPY (▲). The effect of hPP is omitted for clarity because it overlaps the CRC for [Leu$^{31}$,Pro$^{34}$]NPY.

**Table 1** $pIC_{50}$±s.e.mean and equipotent molar ratios (e.p.m.r.), relative to PYY, exhibited by NPY and some analogues in the electrically stimulated isolated vas deferens of rat

| | $pIC_{50}$±s.e. | e.p.m.r. |
|---|---|---|
| PYY | 8.42±0.068 | 1 |
| $NPY_{2-36}$ | 8.16±0.056 | 1.8 |
| NPY | 7.46±0.046 | 9.1 |
| $NPY_{13-36}$ | 6.78±0.054 | 44 |
| $NPY_{18-36}$ | 6.41±0.029 | 102 |
| [Leu$^{31}$-Pro$^{34}$]NPY | 6< | – |
| hPP | 6< | – |

### *Activity of benextramine and PYX-2 in rat vasa deferentia*

BXT at 100 $\mu$M was incubated for 10 min after the control contractile response was stabilized, then the tissues were washed for 15 min, after which ES was reapplied. BXT at 100 $\mu$M produced a small inhibitory effect of the control contractile response which was partially reversed by washing the tissues during the 15 min period of resting. Contractile responses after BXT treatment were reduced by $7.9 \pm 2.7\%$ compared with responses before BXT incubation ($n = 5$).

Preincubation with BXT for 10 min did not affect the CRC to PYY (Figure 2). The estimated $IC_{50}$ value for PYY was $3.59 \pm 0.37$ nM, which was not significantly different from the control value ($3.77 \pm 0.63$ nM; $P = 0.15$ by $F$-test). Increasing the incubation time for BXT to 60 min did not substantially modify the result because the $IC_{50}$ value for PYY was $3.63 \pm 0.30$ nM ($n = 3$; data not shown).

PYX-2 up to 1 $\mu$M was devoid of agonist activity ($n = 4$) and, incubated at 1 $\mu$M for 20 min, with no washouts, was completely ineffective in antagonizing the PYY effect (Figure 3).

### *Activity of suramin on the twitch response and on the smooth muscle response to ATP*

Suramin inhibited the control contractile response (0.15 Hz, 1 ms, 25 V) in a dose-dependent manner with a threshold concentration of 1 $\mu$M. This compound reduced the contractile response by $96.0 \pm 1.1\%$ of the control value at the maximally effective concentration of 100 $\mu$M ($n = 5$). Prazosin (0.1 – 1 $\mu$M) was much less effective; the inhibition reached only $25.4 \pm 5.1\%$ of the control response ($n = 3$). The activity of these drugs is compared in Figure 4.

ATP induced very fast and transient contractions with a threshold concentration of 0.3 $\mu$M. The dose-response curve was not sigmoidal but appeared shallow in the range 0.3 – 300 $\mu$M, steeper in the range of 1000 – 10 000 $\mu$M (Figure 5). A plateau of response could not be reached at the maximal concentration tested (10 000 $\mu$M).

Suramin, at 30, 100 and 300 $\mu$M, shifted to the right the CRC to ATP in a non-competitive manner, because there was no correlation between antagonist dose and shift of CRC to the right; but a dose-related depression of the response at the maximal agonist concentration tested was noted (Figure 5). Thus, it appears that suramin, in this tissue, is a non-competitive antagonist of the purinoceptor activated by ATP on the vas deferens smooth muscle.

### *Effect of NPY on the CRC to ATP*

Incubation of tissues for 2 min with NPY (0.3 $\mu$M) was without effect on the serial dose-response curve to ATP ($n = 6$; data

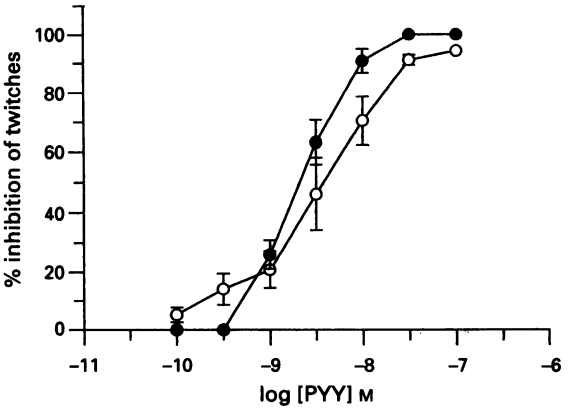


**Figure 3** Effect of PYX-2 on the dose-response curve to PYY in the prostatic portion of the rat isolated vas deferens electrically stimulated with single pulses (25 V, 0.15 Hz, 1 ms). Control curve (○), PYX-2-treated curve (●). Each curve is the mean ± s.e. of 4 experiments carried out on different tissues.

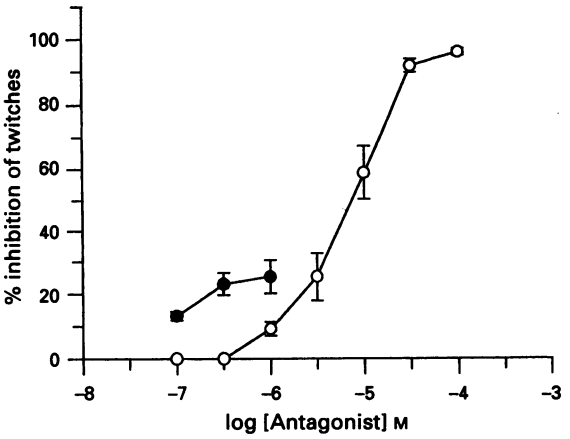


**Figure 4** Inhibitory activity of suramin (○) and prazosin (●) in the prostatic portion of the rat vas deferens electrically stimulated with single pulses (25 V, 0.15 Hz, 1 ms). Each curve is the mean ± s.e. of 3 – 6 experiments carried out on different tissues.

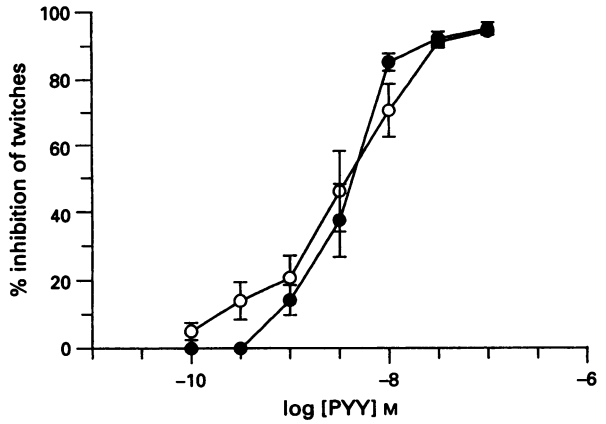


**Figure 2** Lack of effect of benextramine (100$\mu$M for 10 min) on the dose-response curve to PYY in the prostatic portion of the rat isolated vas deferens electrically stimulated with single pulses (25 V, 0.15 Hz, 1 ms). Control curve (○), benextramine-treated curve (●). Each curve is the mean ± s.e. of 5 experiments carried out on different tissues.

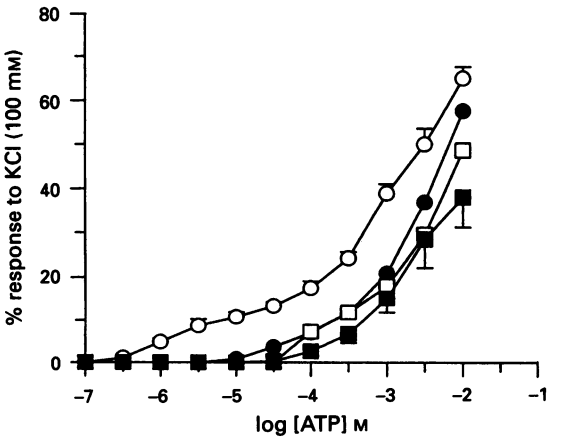


**Figure 5** Dose-response curve to ATP in the prostatic portion of the rat vas deferens in resting conditions and antagonism by different doses of suramin. Controls (○), suramin 30 $\mu$M (●), 100 $\mu$M (□) and 300 $\mu$M (■). Each curve is the mean of 5 experiments carried out on different tissues. The error bars for the dose-response curves in the presence of suramin 30 and 100 $\mu$M are omitted for clarity.

not shown). At this concentration NPY *per se* was devoid of any contractile or relaxant effect on the basal tone of the tissue.

## Discussion

It is well known that in the whole isolated vas deferens of the rat, NPY inhibits contractile responses to single pulse electrical field stimulation by a mechanism involving depression of noradrenaline release from sympathetic nerve terminals (Lundberg & Stjarne, 1984). Later studies proposed that prejunctional inhibition by NPY was mediated exclusively by the $Y_2$ receptor, whereas postjunctional effects of NPY (contraction of vascular smooth muscles and potentiation of noradrenaline contractility) were mediated through the $Y_1$ receptor (Wahlestedt *et al.*, 1986). However, recent findings by some investigators seem to suggest that this is not true for all tissues and animal species (McAuley & Westfall, 1992; Coppes *et al.*, 1994). In particular in rabbit isolated vas deferens, the presence of a prejunctional $Y_1$ receptor, mediating suppression of the twitch response, was proposed (Doods & Krause, 1991). In our experiments in the rat isolated vas deferens, the rank order of agonist potency exhibited by NPY and related peptides strongly supports the existence in this tissue of the $Y_2$ subtype as suggested earlier by other authors (Wahlestedt *et al.*, 1986). This conclusion is supported by the lack of activity of the selective and potent $Y_1$ agonist [Leu$^{31}$,Pro$^{34}$]NPY. In the literature there is some evidence for the existence of the $Y_3$ receptor subtype on which, in contrast to $Y_1$ and $Y_2$ receptors, PYY does not mimic the effects of NPY and may sometimes have the opposite effect (see Wan & Lau, 1995 for a review). The high potency of PYY in our paradigm does not exclude the presence of $Y_3$ receptors but certainly excludes the possibility of the exclusive presence of such a receptor.

The very low efficacy of [Leu$^{31}$,Pro$^{34}$]NPY in our experiments is in contrast with previously published data, using rat isolated prostatic vas deferens, reporting that [Leu$^{31}$,Pro$^{34}$]NPY was only approximately 2–14 times less potent than NPY (Jorgensen *et al.*, 1990; Doods & Krause, 1991). The reason for this discrepancy is not clear but could be due to different experimental conditions, e.g. the longer pulse duration used by Jorgensen *et al.*, 1990 (1s) with respect to our pulse duration (1ms) or the rat strain (Chbb-Thom) used by Doods & Krause (1991) which is different from our strain (Sprague-Dawley).

The presence of two different PP-fold polypeptide receptors in rat vas deferens, one activated by NPY and one by rat PP (rPP), both of which inhibit contractile responses to ES, was suggested in rat vas deferens (Jorgensen *et al.*, 1990). We found that hPP was quite ineffective in inhibiting twitch responses, in contrast to rPP, which was described as equipotent to NPY (Jorgensen *et al.*, 1990). No definitive conclusion about the existence of a prejunctional PP receptor mediating suppression of the twitch response in this preparation can be drawn from our experiments. It is possible that hPP, which differs in 8 amino acids from rPP, has a very low affinity and/or efficacy for the putative PP receptor in the rat vas deferens.

Benextramine is a tetramine disulphide which irreversibly blocks $\alpha_1$ and $\alpha_2$-adrenoceptors with high potency (Melchiorre *et al.*, 1978; Plotek & Atlas, 1983). It was demonstrated that BXT incubated for 30 min at 10 μM followed by 30 min of washing, completely inhibited the smooth muscle contractile response to NA of the whole isolated vas deferens of the rat (Melchiorre *et al.*, 1978). However, it was recently claimed that BXT at high concentrations (200 and 1000 μM) is also able to block selectively $Y_1$ but not $Y_2$ receptors in ligand binding studies performed with rat brain membranes (Doughty *et al.*, 1992). Using the rat isolated femoral artery the same group reported that BXT, incubated for 10 min at 10 μM, antagonized, probably in an irreversible manner, [Leu$^{31}$,Pro$^{34}$]NPY and $NPY_{13-36}$-induced contractions. The authors concluded that BXT is able to block both $Y_1$ and $Y_2$ receptors located on the smooth muscle cell (Tessel *et al.*, 1993). Furthermore, Li *et al.*, 1991 demonstrated that BXT inhibited the specific [$^{125}$I]-NPY labelling of a bovine hippocampal 50 kDa $Y_2$ binding protein with an $IC_{50}$ of 33 μM (Li *et al.*, 1991). More recently, it was reported that BXT inhibited irreversibly two NPY binding sites in the rat brain with $IC_{50}$ values of 29.3 pM and 36.0 μM, respectively (Melchiorre *et al.*, 1994). The authors suggested that the high affinity site for BXT is represented by the $Y_1$ receptor, while the low affinity site is the $Y_2$ receptor. Because data from the literature seem to indicate that BXT is able to antagonize $Y_1$ and $Y_2$ receptors in different tissues and animal species, we decided to test the activity of BXT in rat vas deferens, which in our model seems to express exclusively the $Y_2$ receptor subtype. Two main results were obtained: first, no antagonism of BXT versus PYY was found, even with a concentration of 100 μM and an incubation time of 60 min. This may suggest that the postjunctional $Y_2$ receptor, irreversibly antagonized by BXT in the rat isolated femoral artery, is pharmacologically different from the $Y_2$ receptor, BXT insensitive, described here in the rat isolated vas deferens. Another hypothesis to explain how BXT appears to be a $Y_2$ antagonist in rat femoral artery but not in the vas deferens is related to the well known calcium channel blocking properties of BXT. Because the constriction produced by NPY appears to be at least partially mediated by the influx of extracellular calcium (Walker *et al.*, 1991), it is possible that BXT blocks the NPY-induced contractions in vascular tissues only by this mechanism and not through interaction with NPY receptors. However, it was demonstrated that, in rat isolated femoral artery, BXT at 10 μM, was ineffective against Bay K 8644-induced contractions, but almost completely abolished contractions induced by NPY, [Leu$^{31}$,Pro$^{34}$]NPY and $NPY_{13-36}$, so the hypothesis of $Y_2$ receptor heterogeneity appears to be more probable. However, the use of more specific and competitive $Y_1$ and $Y_2$ receptor antagonists is essential to verify this issue.

The second major finding of our investigation was that BXT decreased only slightly the contractile response to ES even if a dose 10 times higher than that reported to block completely NA contractile response in this tissue (Melchiorre *et al.*, 1978) was used. Furthermore, we were unable to show two phases of the contractile response to a single pulse, as described by others using the rat whole isolated vas deferens (Brown *et al.*, 1979). So we conclude that in our experimental conditions the contractile response is exclusively purinergic. This is consistent with previous investigations demonstrating that in the prostatic portion the twitch response consists mostly or exclusively of a single fast component (McGrath, 1978). Our hypothesis was confirmed by the observation that the selective $P_2$ antagonist, suramin, completely abolished the twitch response in our paradigm. Furthermore, NPY was without effect on the contractile response of the smooth muscle to ATP, so it appears that NPY induces suppression of purinergic neurotransmission only through a prejunctional mechanism, presumably through activation of the $Y_2$ receptor. The nature of the purinergic neurotransmitter involved in the twitch response was not determined in our study. We observed a lack of correlation between doses of suramin and antagonism of ATP-induced contractions, because the right-ward shift obtained with the lowest dose used (30 μM) was very similar to that using suramin at 300 μM. This fact could be due to the interaction of ATP and its products of partial degradation with multiple purinoceptors, some resistant to suramin blockade. In fact it was recently claimed that in this tissue, ATP at 1 mM activates three different purinoceptors namely $P_{2X}$ and $P_{2Y}$, both reported to be fully sensitive to suramin (Cusack, 1993) as well as a novel subtype resistant to suramin (Bultmann & Starke, 1994). Moreover, the irregularly shaped dose-response curve to ATP and the fact that even at the concentration of agonist of 10 mM a plateau of response could not be achieved, precludes the possibility of determining the nature of suramin antagonism.

The peptide PYX-2 was originally proposed by Tatemoto *et al.* (1992) as a NPY antagonist. In fact this compound, up to 1 mM, was devoid of agonist activity but antagonized, in a dose-related manner, the increase in intracellular $Ca^{2+}$ con-

centration induced by 100 nM NPY or PYY in HEL cells, an effect which is thought to be mediated by $Y_1$ receptors (Michel *et al.*, 1990; Feth *et al.*, 1992). In our study on rat isolated vas deferens, however, PYX-2, at the same dose, was completely ineffective in antagonizing the PYY-mediated effect. In the literature contrasting results were obtained with PYX-2 as a $Y_1$ antagonist. In fact, it was recently reported that this compound reduced the magnitude of contractions induced by NPY in both isolated uterine artery and vena cava of the guinea-pig (Morris & Sabesan, 1994). On the other hand, PYX-2 inhibited NPY-induced carbohydrate intake in Sprague-Dawley rats (Leibowitz *et al.*, 1992) but failed to inhibit food intake in obese Zucker rats (Beck *et al.*, 1994). Because there is evidence that NPY is an important mediator of feeding behaviour through a variant of the $Y_1$ receptor (Stanley *et al.*, 1992) it is not clear if PYX-2 is a real antagonist of the $Y_1$ receptor or if it is able to discriminate between two different subtypes of this receptor. Interestingly, we recently characterized a 'non classical' $Y_1$ receptor, located prejunctionally and insensitive to BXT antagonism, in the rabbit isolated vas deferens (Palea *et al.*, 1995). This receptor could be different from that mediating contraction of some isolated vessels, e.g. the rabbit isolated saphenous vein, where the NPY effect is blocked by BXT (Palea *et al.*, 1995). Moreover, in the rabbit isolated vas deferens we found that PYX-2, up to 1 $\mu$M, was devoid of any antagonistic activity versus PYY-induced suppression of the twitch contraction, nor was it a partial agonist (unpublished observation). So, it is possible that PYX-2 could be a selective antagonist for the $Y_1$ receptor subtype found in HEL cells and in some isolated vessels, being ineffective on the $Y_1$ receptor subtype in rabbit isolated vas deferens and possibly also on the putative $Y_1$ receptor described in obese Zucker rats (Beck *et al.*, 1994). However, the peptide nature of PYX-2 and its unknown stability both *in vitro* and *in vivo* do not allow us to draw any definitive conclusion.

In summary, we have demonstrated that the prostatic portion of the rat isolated vas deferens expresses a prejunctional $Y_2$ receptor which could be pharmacologically different from those reported till now in the literature, because of the lack of sensitivity to BXT. Furthermore, we suggest that the contractile response of this tissue to single pulses could be mediated by a purinergic neurotransmitter antagonized by suramin and that NPY could be a potent inhibitory substance for this type of neurotransmission.

## References


ADRIAN, T.E., GU, J., ALLEN, J.M., TATEMOTO, K., POLAK, J.M. & BLOOM, S.R. (1984). Neuropeptide Y in the human male genital tract. *Life Sci.*, **35**, 2643–2648.

BECK, B., STRICKER-KRONGRAD, A., MUSSE, N., NICOLAS, J.P. & BURLET, C. (1994). Putative neuropeptide Y antagonist failed to decrease overeating in obese Zucker rats. *Neurosci. Lett.*, **181**, 126–128.

BULTMANN, R. & STARKE, K. (1994). $P_2$-purinoceptors antagonists discriminate three contraction-mediating receptors for ATP in rat vas deferens. *Naunyn-Schmied. Arch. Pharmacol.*, **349**, 74–80.

BROWN, C.M., MCGRATH, J.C. & SUMMERS, R.J. 1979). The effects of α-adrenoceptor agonists and antagonists on responses of transmurally stimulated prostatic and epididymal portions of the isolated vas deferens of the rat. *Br. J. Pharmacol.*, **66**, 553–564.

COPPES, R.P., SMIT, J., GEURTSEN, A.M.S., ROFFEL, A.D., DAHLOF, C., DOODS, H.N. & ZAAGSMA, J. (1994). Heterogeneity of prejunctional neuropeptide Y receptors inhibiting noradrenaline overflow in the portal vein of freely moving rats. *Eur. J. Pharmacol.*, **261**, 311–316.

CUSACK, N.J. (1993). $P_2$ receptor subclassification and structure activity relationship. *Drug Dev. Res.*, **28**, 244–252.

DELEAN, A., MUNSON, P.J. & RODBARD, D. (1978). Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay and physiological dose-response curves. *Am. J. Physiol.*, **235**, 97–102.

DONOSO, V., SILVA, M., ST-PIERRE, S. & HUIDOBRO-TORO, J.P. (1988). Neuropeptide Y (NPY), an endogenous modulator of adrenergic neurotransmission in the rat vas deferens: structural and functional studies. *Peptides*, **9**, 545–553.

DOODS, H.N. & KRAUSE, J. (1991). Different neuropeptide Y receptor subtypes in rat and rabbit vas deferens. *Eur. J. Pharmacol.*, **204**, 101–103.

DOUGHTY, M.B., LI, K., CHU, S.S. & TESSEL, R. (1992). Benextramine-neuropeptide Y (NPY) binding site interactions: characterization of $^3$H-NPY binding site heterogeneity in rat brain. *Neuropeptides*, **23**, 169–180.

FETH, F., RASCHER, W. & MICHEL, M.C. (1992). Neuropeptide Y (NPY) receptors in HEL cells: comparison of binding and functional parameters for full and partial agonists and a non-peptide antagonist. *Br. J. Pharmacol.*, **105**, 71–76.

FUHLENDORFF, J., GETHER, U., AAKERLUND, L., LANGELAND-JOHANSEN, N., THOGERSEN, H., MELBERG, S.G., OLSEN, U.B., THASTRUP, O. & SCHWARTZ, T.W. (1990). [Leu31-Pro34]Neuropeptide Y: a specific Y1 receptor agonist. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 182–186.

JORGENSEN, J.C., FUHLENDORFF, J. & SCHWARTZ, T.W. (1990). Structure-function studies on neuropeptide Y and pancreatic polypeptide-evidence for two PP-fold receptors in vas deferens. *Eur. J. Pharmacol.*, **186**, 105–114.

KENAKIN, T. (1993). Allotopic, noncompetitive and irreversible antagonism. In *Pharmacologic Analysis of Drug-Receptor Interaction*. pp. 336–342. New York: Raven Press.

LEIBOWITZ, S.F., XUEREB, M. & KIM, T. (1992). Blockade of natural and neuropeptide-Y-induced carbohydrate feeding by a receptor antagonist PYX-2. *Neuroreport*, **3**, 1023–1026.

LI, W., MACDONALD, R.G. & HEXUM, T.D. (1991). Benextramine irreversibly inhibits [$^{125}$I] neuropeptide Y affinity labeling of the Y2 binding protein in bovine hippocampus. *Eur. J. Pharmacol. Mol. Pharmacology Section*, **207**, 89–91.

LUNDBERG, J.M. & STJARNE, L.V. (1984). Neuropeptide Y (NPY) depresses the secretion of $^3$H-noradrenaline and the contractile response evoked by field stimulation, in rat vas deferens. *Acta Physiol. Scand.*, **120**, 477–479.

MALLARD, N., MARSHALL, R., SITHERS, A. & SPRIGGS, B. (1992). Suramin: a selective inhibitor of purinergic neurotransmission in the rat isolated vas deferens. *Eur. J. Pharmacol.*, **220**, 1–10.

MCAULEY, M.A. & WESTFALL, T.C. (1992). Possible location and function of neuropeptide Y receptor subtypes in the rat mesenteric arterial bed. *J. Pharmacol. Exp. Ther.*, **261**, 863–868.

MCGRATH, J.C. (1978). Adrengeric and 'non-adrenergic' components in the contractile response of the vas deferens to a single indirect stimulus. *J. Physiol.*, **283**, 23–29.

MELCHIORRE, C., ROMUALDI, P., BOLOGNESI, M.L., DONATINI, A. & FERRI, S. (1994). Binding profile of benextramine at neuropeptide Y receptor subtypes in rat brain areas. *Eur. J. Pharmacol.*, **265**, 93–98.

MELCHIORRE, C., YONG, M.S., BENFEY, B.G. & BELLEAU, B. (1978). Molecular properties of the adrenergic α receptor. 2. Optimum covalent inhibition by two different prototypes of polyamine disulfides. *J. Med. Chem.*, **21**, 1126–1132.

MICHEL, M.C., SCHLICKER, E., FINK, K., BOUBLIK, J.H., GOTHERT, M., WILLETTE, R.N., DALY, R.N., HIEBLE, J.P., RIVIER, J.E. & MOTULSKY, H.J. (1990). Distinction of NPY receptors in vitro and in vivo I. NPY(18–36) discriminates receptor subtypes in vitro. *Am. J. Physiol.*, **259**, E131–E139.

MORRIS, J.L. & SABESAN, S. (1994). Comparison of the NPY receptors mediating vasoconstriction of the guinea-pig uterine artery and thoracic vena cava using a range of NPY analogues. *Neuropeptides*, **26**, 21–28.

PALEA, S., RIMLAND, J.M., CORSI, M. & TRIST, D.G. (1994). Evidence for the presence of an atypical prejunctional NPY $Y_1$ receptor in rabbit isolated vas deferens: comparison with the rat vas deferens. *Br. J. Pharmacol.*, **112**, 569P.

PALEA, S., CORSI, M., RIMLAND, J.M. & TRIST, D.G. (1995). Discrimination by benextramine between the NPY-$Y_1$ receptors subtypes present in rabbit isolated vas deferens and saphenous vein. *Br. J. Pharmacol.*, **115**, 3–10.

PLOTEK, Y. & ATLAS, D. (1983). Characterization of benextramine as an irreversible α-adrenergic blocker and as a blocker of potassium-activated calcium channels. *Eur. J. Biochem.*, **133**, 539–544.

STANLEY, B.G., MAGDALIN, W., SEIRAFI, A., NGUYEN, M.M. & LEIBOWITZ, S.F. (1992). Evidence for neuropeptide Y mediation of eating produced by food deprivation and for a variant of the $Y_1$ receptor mediating this peptide effect. *Peptides*, **13**, 581–587.

STJARNE, L., LUNDBERG, J.M. & ÅSTRAND, P. (1986). Neuropeptide Y- a cotransmitter with noradrenaline and adenosine 5'-triphosphate in the sympathetic nerves of the mouse vas deferens? A biochemical, physiological and electropharmacological study. *Neuroscience*, **18**, 151–166.

TATEMOTO, K., MANN, M. & SHIMIZU, M. (1992). Synthesis of receptor antagonists of neuropeptide Y. *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 1174–1178.

TESSEL, R., MILLER, D.W., MISSE, G.A., DONG, X. & DOUGHTY, M.B. (1993). Characterization of vascular postsynaptic neuropeptide Y receptor function and regulation. 1. NPY-induced constriction in isolated rat femoral artery rings is mediated by both Y1 and Y2 receptors: evidence from benextramine protection studies. *J. Pharmacol. Exp. Ther.*, **265**, 172–177.

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

WALKER, P., DROUZMAN, E., BURNIER, M. & WAEBER, B. (1991). The role of neuropeptide Y in cardiovascular regulation. *Trends Pharmacol. Sci.*, **12**, 111–115.

WAN, C.P. & LAU, B.H.S. (1995). Neuropeptide Y receptor subtypes. *Life Sci.*, **56**, 1055–1064.

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