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# The effects of neuropeptide Y and its fragments upon basal and electrically stimulated ion secretion in rat jejunum mucosa

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**1** The effects of neuropeptide Y (NPY) and a range of C terminal fragments were investigated both on basal short circuit current (s.c.c.) and electrical field stimulated responses in voltage clamped preparations in rat jejunal mucosa.

**2** Most of the NPY fragments tested had direct effects upon the mucosa, reducing baseline s.c.c. with $EC_{50}$ values of 1 $\mu$M or more. NPY was 30 times more effective than any of the fragments tested and the order of potency was: NPY $\gg$ NPY (11–36) $\geqslant$ (12–36) $\geqslant$ (13–36) $\geqslant$ (14–36). NPY (15–36), (16–36), (20–36) and (22–36) were still less effective and complete concentration-response curves could not be constructed. NPY (26–36), des amido NPY and the C-terminal flanking peptide of NPY (CPON) were all inactive and did not significantly alter responses to NPY.

**3** Electrical field stimulation (EFS) of mucosal preparations elicited rapid transient secretory responses in the presence of hexamethonium and atropine. NPY and fragments attenuated these secretory responses and where concentration-response relationships could be compared at a given time point the following order of potency was obtained: NPY $\gg$ NPY (11–36) > NPY (13–36). Again NPY (26–36), des amido NPY and CPON were ineffective, while at single concentrations (300 nM) a graded attenuation of EFS responses was obtained with NPY (14–36) $\geqslant$ NPY (15–36) > NPY (16–36) $\geqslant$ NPY (20–36) > NPY (22–36).

**4** The attenuation of EFS responses by these peptides was not dependent upon the basal secretory state. Pretreatment of tissues with piroxicam reduced s.c.c. and attenuated further reductions in s.c.c. by NPY, but had no effect upon NPY-mediated inhibition of electrically-stimulated secretory responses.

**5** NPY fragments attenuated both basal and EFS generated secretion. Since fragments are effective these receptors must, by definition be $Y_2$-like. NPY (11–36) and NPY (13–36) at 300 nM and 1 $\mu$M did not significantly attenuate secretory responses to either carbachol (CCh) or substance P (SP). A 1 $\mu$M concentration of either fragment was equivalent in effect to 30 nM NPY upon basal current, but NPY at this concentration significantly reduced both CCh- and SP-induced secretion. The reduced spectrum of fragment activity together with the different order and potency ratios obtained with these three peptides indicates a presynaptic action for NPY and the fragments.

## Introduction

Neuropeptide (NPY) is located in subpopulations of both myenteric and submucous enteric neurones in the rat small intestine (Ekblad *et al.*, 1987). In addition extrinsic perivascular fibres also contain NPY, these being sympathetic in origin (Sundler *et al.*, 1983). Intrinsic submucous and myenteric NPY-positive neurones also contain vasoactive intestinal polypeptide (VIP) (Ekblad *et al.*, 1984; 1987) and whilst colocalisation of these two peptides is also observed in other systems of both rat and man (Leblanc *et al.*, 1987; Wattchow *et al.*, 1987), it does not appear to be a common pairing in the guinea-pig (Furness *et al.*, 1985). In the rat small intestine, VIP/NPY positive neurones in the myenteric ganglia issue short (2 mm) anal projections, whilst submucous VIP/NPY neurones project longer distances (4 mm) in an oral direction and it is these fibres that predominantly innervate the mucosa (Ekblad *et al.*, 1987). The abundant innervation of smooth muscle, vascular elements and mucosa by NPY-containing intrinsic neurones implicate this neuropeptide as a modulator of several enteric functions.

Both pre- and postjunctional NPY effects have been observed at sympathetic neuroeffector junctions (Wahlestedt *et al.*, 1986). In addition to direct vasoconstriction, NPY and its structural analogue peptide YY (PYY) also potentiated the postjunctional effect of noradrenaline (NA), as well as attenuating NA release. PYY derives from a different precursor to NPY, but like NPY it is 36 amino acids long and exhibits 70% sequence homology with NPY. The prejunctional inhibition of NA release was also observed with the C-terminal fragment PYY (13–36) which had no effect postjunctionally. Thus, the presence of two different NPY receptors was postulated, $Y_1$ being postjunctional and accepting only full length NPY and PYY, and $Y_2$ prejunctional receptors that could also be activated by fragments of NPY and PYY (Wahlestedt *et al.*, 1987). However, in the splenic vascular bed, NPY (13–36) also exerted postjunctional vasoconstrictor activity (Lundberg *et al.*, 1988) and shorter fragments NPY (16–36) and NPY (19–36) also exhibited direct vasoconstrictor effects in the guinea-pig coronary circulation *in vitro* (Rioux *et al.*, 1986). Thus in these two systems $Y_2$-like receptors appear to be located within postjunctional membranes. The use of a subdivided classification of NPY receptors is becoming widespread. However, $Y_1$-receptors, which will only accept full length NPY, are not always postjunctional and $Y_2$-receptors, which will tolerate loss of N terminal residues of NPY, are not necessarily prejunctional.

In the rat small intestine NPY is a potent antisecretory agent, inhibiting both basal Cl secretion (Cox *et al.*, 1988) and attenuating secretory responses generated by a range of neuropeptides and neurotransmitters (Cox & Cuthbert, 1988). Early preliminary data appeared to suggest that NPY (13–36) did not alter basal short circuit current (s.c.c.) while human pancreatic polypeptide was found to be considerably less potent than NPY and PYY (Cox *et al.*, 1988). Under voltage clamped conditions, mucosal preparations can be electrically stimulated (Hubel, 1978; Cooke *et al.*, 1983) and the resultant secretory responses (predominantly due to Cl secretion) are tetrodotoxin (TTX)-sensitive but not entirely cholinergic in character (Hubel, 1984; Cooke, 1984; Keast *et al.*, 1985). Much of this work has utilized rabbit and guinea-pig small intestine with little reference to the rat intestine. Nevertheless,

candidates for the atropine resistant component of electrically-stimulated secretory responses include substance P (Keast *et al.*, 1985) and possibly VIP (Gaginella *et al.*, 1981), though the pharmacological tools to verify this are still lacking.

The aim of this study was to establish what effects NPY had specifically upon non-cholinergic, electrically-stimulated secretory responses in rat jejunum mucosal preparations, containing intact submucous ganglia. The effects of a range of NPY fragments were also investigated upon both basal and electrically-stimulated epithelial anion secretion, in an attempt to identify the receptors and their location.

## Methods

### *Electrical field stimulation studies*

Epithelial sheets of jejunum were prepared from male Sprague-Dawley rats (200–300 g) as described previously (Cox *et al.*, 1988). Overlying smooth muscle layers were removed by dissection and the remaining mucosal sheets were placed between two halves of perspex Ussing chambers. The chambers accommodated two silver sheet electrodes both of which contacted the serosal surface of the mucosa. The exposed area of tissue was 0.6 $cm^2$ and preparations were automatically voltage clamped with WPI DVC 1000 voltage clamps. Under these conditions changes in short circuit current (s.c.c.) were recorded automatically. Four preparations from adjacent areas were routinely set up from a single jejunum, and all exhibited comparable basal s.c.c. and potential differences. Tissues were bathed in Krebs-Henseleit solution with the following composition (mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 24.8, $KH_2PO_4$ 1.2 and glucose 11.1, and were gassed with 95% $O_2$/5% $CO_2$.

The electrodes were attached to the output of a Tektronix pulse generator which delivered rectangular pulses of 0.6 ms duration and frequency of 5 Hz. Trains of 5 stimuli were delivered every 7 min throughout the experiment once a steady baseline had been achieved. After 4 trains had been delivered hexamethonium, 10 $\mu$M (Hex) and atropine, 10 $\mu$M (Atr) were added and a further 4 trains delivered. The mean value of these latter four responses was denoted as 100% for that preparation. Because of the variation in size of electrical field stimulation (EFS) responses between tissues, values are quoted as percentages of control responses obtained in a particular preparation. Trains of stimuli were continued at 7 min intervals following serosal application of either NPY or one of its fragments. Recording was continued until responses returned to the original, 100%, control levels. Unless otherwise stated EFS responses are given as a percentage of the control response obtained in the presence of Hex and Atr. Changes in baseline s.c.c. are quoted as $\mu$A 0.6 $cm^{-2}$, mean ± 1 s.e.mean. When the effects of peptides upon the secretory responses generated by EFS are compared, experimental values are quoted as percentages of controls, again as mean ± s.e.mean. Unpaired Student's *t* test was used for the statistical analysis of data (unless otherwise stated) and a *P* value of less than 0.05 was considered statistically significant.

### *Materials*

Full length NPY (1–36) together with NPY (13–36), (16–36), (20–36), (22–36), (26–36) and the C-terminal flanking peptide of NPY (CPON) were purchased from Peninsula Laboratories Europe Ltd. Other NPY fragments namely NPY (11–36), (12–36), (13–36), (14–36) and (15–36) were gifts from Prof. Rolf Håkanson, Department of Pharmacology, Lund University. Hexamethonium, atropine, piroxicam and TTX were obtained from Sigma, Poole and all other reagents were of analytical grade.

## Results

The effects of NPY and the C-terminal fragments of NPY upon baseline s.c.c. are shown in Figure 1. Clearly full length

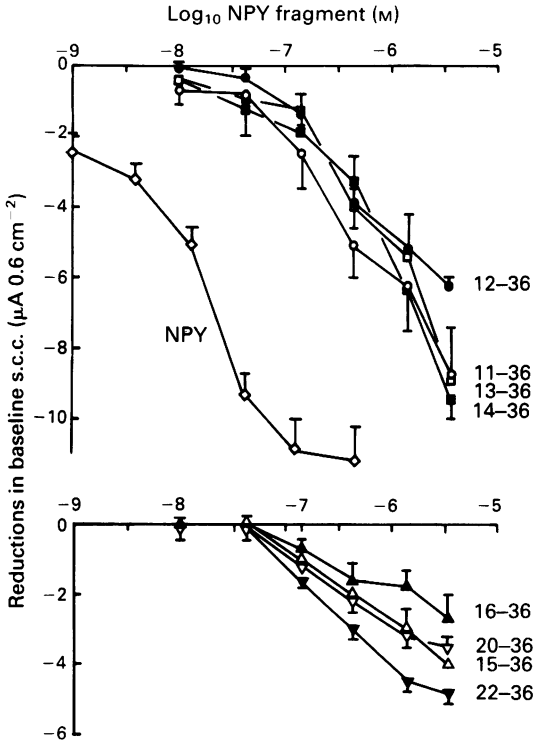


**Figure 1** Antisecretory effects of neuropeptide (NPY) and fragments upon baseline short circuit current (s.c.c.). All peptide additions were made to the basolateral surface in a cumulative fashion and reductions in s.c.c. were recorded as $\mu$A 0.6 $cm^{-2}$. Each point represents the mean with vertical lines indicating 1 s.e.mean (some errors are omitted for reasons of clarity). The number of observations for each fragment are as follows: NPY, 3–11; NPY (11–36), 3–9; NPY (12–36), 2–9; NPY (13–36), 2–11; NPY (14–36), 2–9; NPY (15–36), 2–7; NPY (16–36), 2–7; NPY (20–36), 3–7; NPY (22–36), 3–7.

NPY is considerably more potent than any of the fragments tested with an $EC_{50}$ of 13 nM. Shorter fragments of NPY were also antisecretory but were less potent; NPY (11–36), (12–36), (13–36) and (14–36) had threshold concentrations of 10–30 nM and approximate $EC_{50}$ values of 2 $\mu$M. These fragments would appear to be full agonists. NPY (15–36), (16–36), (20–36) and (22–36) were less potent still with threshold concentrations of 30–100 nM. At the highest possible concentration (3 $\mu$M) tested these four fragments reduced baseline current by between −3.0 and −5.0 $\mu$A 0.6 $cm^{-2}$. Thus it was not possible to determine whether these fragments were full or only partial agonists. NPY (26–36), des amido NPY and CPON were without effect on baseline s.c.c. and had no significant effect upon NPY antisecretory responses. In the presence of either 300 nM NPY (26–36), des amido NPY or CPON, 30 nM NPY reduced s.c.c. by −6.5 ± 1.3 $\mu$A 0.6 $cm^{-2}$ ($n = 6$), −7.9 ± 1.4 $\mu$A 0.6 $cm^{-2}$ ($n = 8$) and −6.9 ± 0.6 $\mu$A 0.6 $cm^{-2}$ ($n = 6$) respectively, compared with controls of −8.6 ± 1.5 $\mu$A 0.6 $cm^{-2}$ ($n = 5$).

EFS (5 Hz, 0.6 ms for 1 s) elicited rapid transient increases in s.c.c. that returned to the baseline within 3 min. These secretory responses were constant when stimuli were applied at 7 min intervals and were attenuated 50% by simultaneous basolateral addition of Hex and Atr (from controls of 7.6 ± 0.6 $\mu$A 0.6 $cm^{-2}$ ($n = 79$) to 3.8 ± 0.3 $\mu$A 0.6 $cm^{-2}$ ($n = 79$)). Subsequent application of 100 nM TTX inhibited EFS responses further to 0.6 ± 0.1 $\mu$A 0.6 $cm^{-2}$, $n = 12$.

Following pretreatment of tissues with Hex and Atr either NPY or one of the NPY fragments was applied and changes in the baseline current and EFS responses recorded. NPY (3 nM and 10 nM) reduced the baseline s.c.c. as expected (Figure 2 and Cox *et al.*, 1988) and also attenuated secretory EFS responses in a concentration-dependent manner. NPY, 1 nM did not significantly inhibit either the basal s.c.c. or EFS responses. The apparent $EC_{50}$ for NPY was 3 nM by inter-

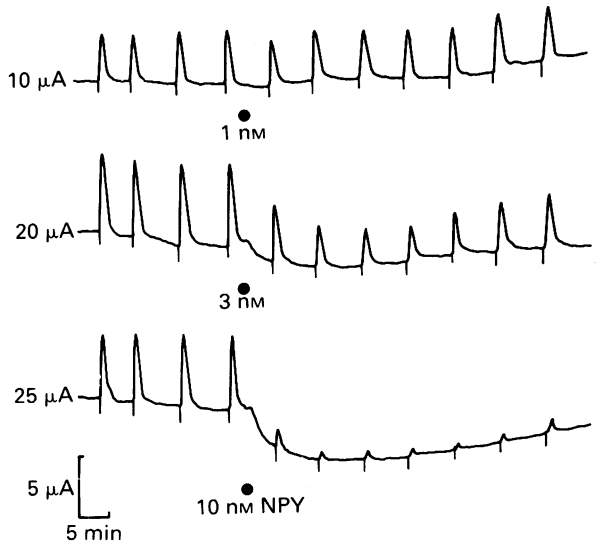


**Figure 2** The effect of neuropeptide (NPY) upon electrical field stimulated (EFS) responses in the presence of hexamethonium and atropine (each 10 μM). Stimuli (5 Hz, 0.6 ms for 1 s) were delivered at 7 min intervals. Application of hexamethonium and atropine reduced EFS responses 40% (not shown) and the remaining resistant secretory responses were inhibited in a concentration-dependent manner by NPY (applied basolaterally). Baseline currents are given on the left and these three traces were obtained from adjacent sections of jejunum.

polation of the results at 14 min (as shown in Figure 3). Pooled data for NPY are compared with that of NPY (13–36) and des amido NPY in Figure 3. Higher concentrations (i.e. 100 nM) of NPY (13–36) were necessary to affect an inhibition of EFS responses similar to 3 nM NPY. NPY (13–36) (30 nM) did not significantly alter basal s.c.c. ($-0.4 \pm 0.3$ μA 0.6 cm$^{-2}$, $n = 4$), while 100 nM NPY (13–36) significantly reduced s.c.c. by $-1.4 \pm 0.6$ μA 0.6 cm$^{-2}$ ($n = 7$, $P < 0.05$). Interpolation of data in the same way as for NPY, gave an approximate $EC_{50}$ of 200 nM for NPY (13–36). A graded inhibition of EFS responses was also obtained with increasing NPY (11–36) concentrations from 10–300 nM. Fourteen minutes after addition of this fragment EFS responses were attenuated by 30%, 57% 83% and 89% with 10 nM, 30 nM, 100 nM and 300 nM NPY (11–36) respectively, allowing an $EC_{50}$ estimation of 40 nM. After 56 min responses had returned to controls in 10 and 30 nM NPY (11–36)-treated preparations, but they were still reduced (by 15% and 89%) in tissues that had received 100 and 300 nM NPY (11–36). Loss of the C-terminal amino group of NPY, i.e. des amido NPY, resulted in total loss of activity, there being no change in either basal or EFS generated s.c.c.

The same range of fragments was tested for their ability to attenuate secretory responses generated by EFS. A concentration of 300 nM was chosen in each case, because measurable changes in baseline s.c.c. were recorded at this concentration and the responses obtained were on the linear portion of each respective concentration-response relationship (Figure 1). Figure 4a and b show the time-dependent attenuation of EFS responses in the presence of each NPY fragment tested. NPY (12–36), (13–36), (14–36) and (15–36) appeared to be equipotent with each other (at 300 nM), reducing field stimulated secretion by 60% 7–21 min after fragment addition; responses recovering within the experimental period (Figure 4a). NPY (16–36) and NPY (20–36) were slightly less effective, reducing EFS responses to $70.3 \pm 4.8\%$ ($n = 4$) and $55.2 \pm 6.0\%$ ($n = 4$) of controls respectively after 7 min (Figure 4b). With NPY

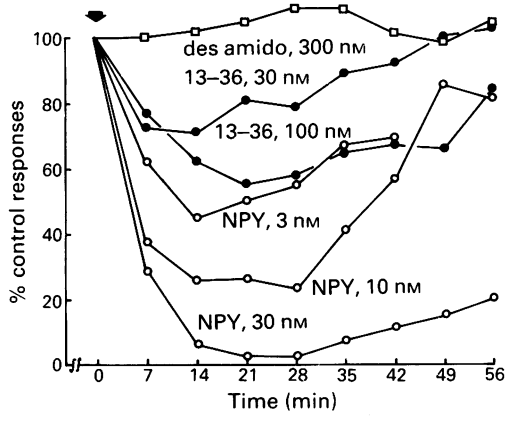


**Figure 3** Pooled data comparing the inhibitory effects of neuropeptide Y (NPY), NPY (13–36) and des amido NPY upon electrical field stimulated (EFS) responses in the presence of hexamethonium and atropine. Control non-cholinergic responses were obtained in each tissue before the application of NPY and designated 100% ($4.5 \pm 0.8$ μA 0.6 cm$^{-2}$ $n = 12$ for 'NPY' controls; $3.1 \pm 0.6$ μA 0.6 cm$^{-2}$, $n = 4$ for 'des amido NPY' controls and $2.2 \pm 0.3$ μA 0.6 cm$^{-2}$, $n = 11$ for NPY (13–36) controls). One of the three peptides was then added basolaterally and all subsequent secretory EFS responses were calculated as a percentage of their respective tissue controls. Concentrations are given as nM and $n$ values were 3, 4 and 3 for 3, 10 and 30 nM NPY; $n = 4$ and 7 for 30 and 100 nM NPY (13–36) and $n = 4$ for 300 nM des amido NPY, respectively. All points represent the mean and errors are not shown, though they were 5–10% of respective means.

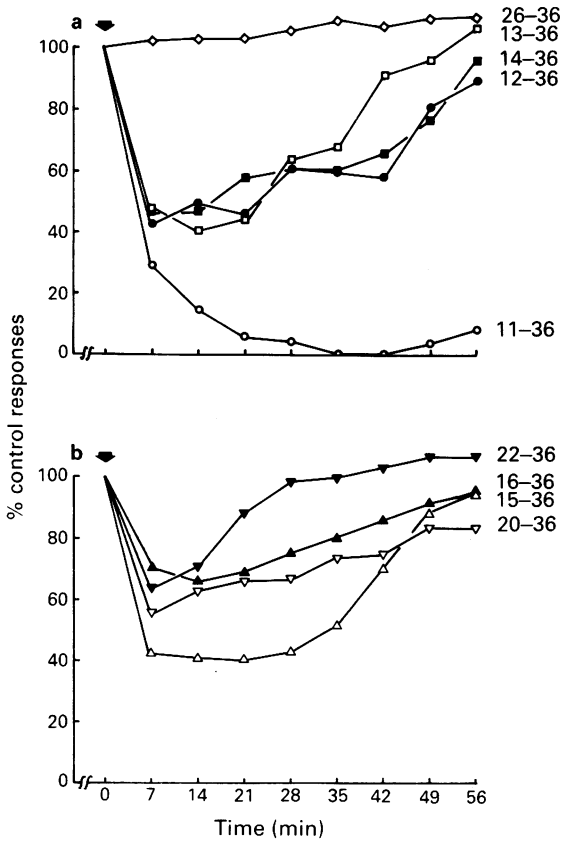


**Figure 4** The effect of a range of neuropeptide Y (NPY) fragments upon hexamethonium/atropine-resistant electrical field stimulated (EFS) responses. Control secretory responses were obtained in each tissue in the presence of hexamethonium and atropine before application of NPY fragments (at 300 nM throughout). The figure is split into (a) and (b) for reasons of clarity. Control EFS responses were denoted as 100% and all subsequent increases in s.c.c. after EFS were calculated as a percentage of each tissue control. Control EFS responses as μA 0.6 cm$^{-2}$ were as follows with $n$ values in parentheses: $2.9 \pm 0.4$ (4) for NPY (11–36); $1.7 \pm 0.2$ (3) for NPY (12–36); $1.8 \pm 0.2$ (4) for NPY (13–36); $3.4 \pm 0.8$ (4) for NPY (14–36); $5.5 \pm 0.8$ (4) for NPY (15–36); $6.5 \pm 0.5$ (4) for NPY (16–36); $4.7 \pm 0.3$ (4) for NPY (20–36); $6.9 \pm 1.7$ (4) for NPY (22–36) and $6.6 \pm 0.8$ (4) for NPY (26–36). All points and values are quoted as means and the errors are not shown in the figure for reasons of clarity, but were not greater than 10%.

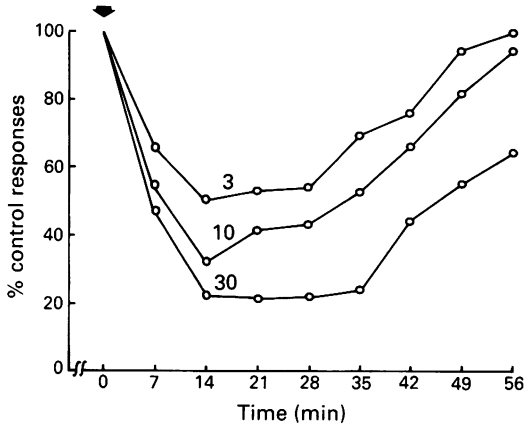


**Figure 5** The effect of 3, 10 and 30 nM neuropeptide Y (NPY) (1–36) upon electrical field stimulated (EFS) responses in the absence of hexamethonium and atropine. Control responses to field stimulation were obtained initially in each tissue before basolateral application of NPY. Controls were $8.0 \pm 2.6\,\mu A\ 0.6\,cm^{-2}$ (3), $9.5 \pm 1.7\,\mu A\ 0.6\,cm^{-2}$ (3) and $4.7 \pm 1.1\,\mu A\ 0.6\,cm^{-2}$ (3) for 3, 10 and 30 nM NPY respectively. Values are quoted as means ± s.e.mean with $n$ values in parentheses. Points in the figure are means but errors are not shown for reasons of clarity, though they were routinely 10–15% of respective mean values.

(22–36) inhibition was of short duration, the responses were reduced to 63.1 ± 6.2% ($n = 4$) of controls after 7 min and returned to control size within 28 min. As before with baseline s.c.c. changes, NPY (26–36) was inactive, eight consecutive trains of stimuli were not altered in size compared with those obtained before fragment addition.

Neither NPY (11–36) nor NPY (13–36) at concentrations of 300 nM, significantly altered secretory responses generated by exogenous carbachol (CCh). In the absence of either fragment 10 μM CCh (a submaximal concentration) increased s.c.c. by $57.5 \pm 6.4\,\mu A\ 0.6\,cm^{-2}$ ($n = 12$) compared with $43.7 \pm 4.4\,\mu A\ 0.6\,cm^{-2}$ in the presence of NPY (13–36) ($n = 11$, $P < 0.1$) and $49.4 \pm 9.2\,\mu A\ 0.6\,cm^{-2}$ with NPY (13–36) ($n = 4$, $P < 0.5$). NPY (11–36) (300 nM) did not alter submaximal substance P (SP 100 nM) secretory responses either (controls were $24.3 \pm 4.3\,\mu A\ 0.6\,cm^{-2}$, $n = 6$ and with NPY (11–36) $15.7 \pm 4.3\,\mu A\ 0.6\,cm^{-2}$, $n = 6$, $P < 0.3$). Paired $t$ test analysis did not yield significant differences between CCh and SP groups in the absence or presence of either NPY (11–36) or NPY (13–36) respectively. Only the inhibition afforded by NPY (13–36) upon CCh secretory responses was significant ($P < 0.05$) by one tailed, paired $t$ test. The effects of 1 μM NPY (13–36) and 10 nM NPY pretreatment (concentrations chosen because they cause similar reductions in basal s.c.c., Figure 1) on SP (100 nM) responses were also compared. Control secretory responses to SP were $29.6 \pm 3.6\,\mu A\ 0.6\,cm^{-2}$ ($n = 10$), while in the presence of NPY (10 nM) they were significantly reduced to $17.6 \pm 1.9\,\mu A\ 0.6\,cm^{-2}$ ($n = 8$, $P < 0.05$), and in the presence of NPY (13–36) (1 μM) $19.2 \pm 3.7\,\mu A\ 0.6\,cm^{-2}$ ($n = 6$, $P < 0.1$).

In all the field stimulation experiments described above the tissues were pretreated with Hex and Atr in order to establish what effects NPY and its fragments had exclusively upon non-cholinergic-mediated ion secretion. In a separate series the effects of NPY upon combined cholinergic and non-cholinergic EFS responses were investigated in the absence of blocking agents (Figure 5). There was no apparent difference in the extent to which 3, 10 and 30 nM NPY attenuated EFS responses either in the presence or the absence of cholinoceptor antagonists. The only significant difference was observed at 28 min after addition of 30 nM NPY. In the presence of the antagonists EFS responses were 2.5 ± 2.5% of controls ($n = 3$), while in the absence of Hex and Atr responses were 22.0 ± 0.3% ($n = 3$, $P < 0.01$) and there appeared to be a more rapid recovery of EFS responses in the latter group.

To investigate whether the lowering of baseline s.c.c. determined the size of the EFS responses, 5 μM piroxicam (a cyclooxygenase inhibitor) was added to both tissue surfaces (Figure 6) with a resultant reduction in s.c.c. of $-15.0 \pm 1.0\,\mu A\ 0.6\,cm^{-2}$ ($n = 7$; from $32.8 \pm 3.7\,\mu A\ 0.6\,cm^{-2}$ to $18.8 \pm 3.9\,\mu A\ 0.6\,cm^{-2}$). EFS responses (in the absence of Hex and Atr) were not changed following piroxicam ($6.3 \pm 1.2\,\mu A\ 0.6\,cm^{-2}$, $n = 7$) compared with those obtained before piroxicam pretreatment ($7.1 \pm 1.2\,\mu A\ 0.6\,cm^{-2}$, $n = 7$). Application of NPY subsequent to piroxicam did not further reduce the s.c.c. (as seen previously, Cox *et al.*, 1988 and Cox & Cuthbert, 1988). However, EFS responses were attenuated, by 16.4 ± 2.6% ($n = 2$), 77.3 ± 4.9% ($n = 3$) and 85.4 ± 4.4% ($n = 3$), 14 min after application of 3, 10 and 30 nM NPY respectively. These data were similar to NPY inhibition in the absence of piroxicam, at 14 min, 3, 10 and 30 nM NPY inhibited EFS responses by 49.4 ± 17.6% ($n = 3$), 68.0 ± 11.6% ($n = 3$) and 81.6 ± 9.3% ($n = 3$), respectively.

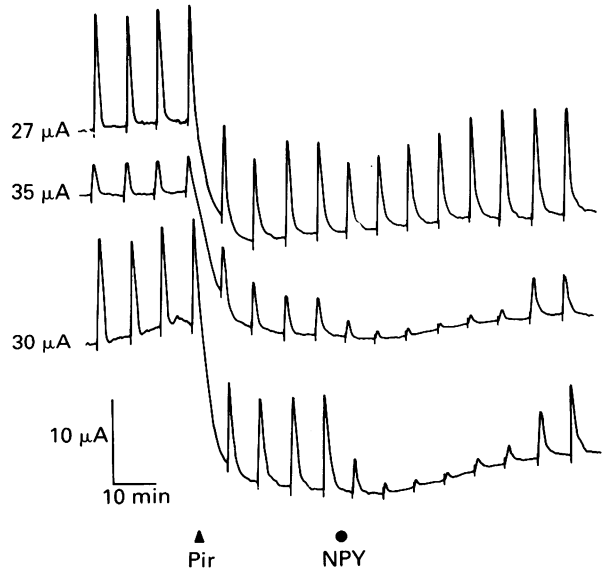


**Figure 6** Effect of piroxicam and neuropeptide Y (NPY) upon electrical field stimulated (EFS) responses. All tissues received 5 μM piroxicam (Pir) both apically and basolaterally and once the baseline short circuit current had reached a steady level NPY 3 nM (upper trace), 10 nM (middle) and 30 nM (lower trace) was applied.

## Discussion

In this study the effects of NPY and a range of NPY fragments were tested both on baseline jejunal s.c.c. and electrically stimulated secretory responses. In both cases full length NPY was more effective than any of the fragments as an inhibitor of both baseline and electrically stimulated s.c.c. The order of potency observed for attenuation of basal s.c.c. was NPY ≫ NPY (11–36), (12–36), (13–36), (14–36). The possibility that fragments (NPY) (15–36), (16–36), (20–36) and (22–36) may be partial agonists should not be overlooked in producing agonist orders of potency (Kenakin, 1983). However, within the concentration range tested the longer fragments NPY (11–36), (12–36), (13–36) and (14–36) all appeared to be full agonists, indicating that on epithelial membranes NPY receptors would appear to be of the $Y_2$ type. This is in agreement with biochemical studies of Servin *et al.* (1989) who, using epithelial cells from rat jejunum, found that C terminal fragments of NPY could inhibit specific [$^{125}$I]-PYY binding and attenuate VIP stimulated adenosine 3′:5′-cyclic monophosphate (cyclic AMP) production.

Residues 7–17 in the NPY molecule are thought to be important structurally, stabilizing the peptide in a conformation favourable for receptor interaction (Krstenansky *et al.*, 1989), while the C terminal region of NPY, 14–30 exhibits the potential to form an amphiphilic α-helix (Krstenansky &

Buck, 1987). Thus loss of amino acids from this region may well result in disruption and unfolding of the molecule thereby further reducing peptide efficacy. Amino acids 11, 12, 13 and 14 form the core sequence holding together the N terminal polyproline helix and amphiphilic α-helix of the C terminal (as shown in Fuhlendorff *et al.*, 1990). Sequential loss of these residues should therefore not greatly alter the tertiary structure of the remaining peptide. Although NPY (11–36), (12–36), (13–36) and (14–36) were found to be significantly less potent than NPY itself, these four fragments were equipotent at reducing baseline s.c.c. However, NPY fragments shorter than these i.e. NPY (15–36), (16–36), (20–36) and (22–36) were less effective again, indicating the importance of the full α-helix in peptide-receptor recognition.

The C terminal decapeptide, NPY (26–36), plus des amido NPY and CPON were all inactive, affecting neither the baseline s.c.c. nor EFS responses and not altering NPY responses in any way. Although a product of the same precursor as NPY, CPON appears to have little or no biological activity in other systems (Potter *et al.*, 1989). Equally, loss of the C-terminal amide group from full length NPY results in a loss of biological activity as shown above and elsewhere (Wahlestedt *et al.*, 1986; Potter *et al.*, 1989; MacKerell *et al.*, 1989). A noticeable difference in the ability to reduce s.c.c. and to inhibit EFS was seen with NPY (11–36). This peptide was equipotent with NPY (12–36), (13–36) and (14–36) at reducing basal s.c.c. (with an approximate $EC_{50}$ of 2 μM). However, it was significantly more effective than the shorter fragments at inhibiting EFS responses ($EC_{50}$ of 40 nM).

The size of EFS responses was not dependent upon the basal s.c.c., since piroxicam had no significant effect upon EFS secretory responses. However, subsequent application of NPY still attenuated EFS responses to a similar extent as in the absence of piroxicam. NPY attenuated both cholinergic and non-cholinergic components of EFS secretory response in rat small intestine. Direct prejunctional inhibition of both cholinergic and non-cholinergic induced contraction of small intestinal circular smooth muscle has been observed in the guinea-pig (Holzer *et al.*, 1987), whereas in guinea-pig colon longitudinal muscle NPY attenuates cholinergic transmission via an α-adrenergic pathway (Wiley & Owyang, 1987). McCulloch *et al.* (1987) observed NPY attenuation of field stimulated and muscarinic agonist-stimulated chloride secretion in guinea-pig distal colon. However, in this preparation NPY had no effect upon the basal secretory state of the tissue. In the rat jejunum NPY attenuates not only secretory responses generated electrically but also those observed following addition of exogenous CCh, SP and VIP (Cox & Cuthbert, 1988). Both CCh and SP stimulate Cl secretion via $Ca^{2+}$-dependent mechanisms, while VIP stimulates intracellular cyclic AMP generation thereby increasing Cl secretion. NPY (and presumably the gastrointestinal hormone and structural analogue, PYY) significantly attenuated CCh and VIP responses and abolished SP-induced secretion. Thus, the possibility that NPY fragments might similarly alter epithelial responses via postjunctional receptors had to be investigated.

Conventional statistical analysis (unpaired Student's *t* test) showed that 300 nM NPY (11–36) and NPY (13–36) did not significantly alter either CCh or SP secretory responses. Even when data were paired from each group (which is not routinely done) and analysed by two-tailed *t* tests, no significant differences were found. The only marginal difference ($P < 0.05$) was seen with 300 nM NPY (13–36), which with a single-tailed paired analysis was shown to attenuate CCh responses significantly. A comparison was also made between the effects of 10 nM NPY and 1 μM NPY (13–36), both of which attenuated basal s.c.c. by approximately 50%. Whereas pretreatment of preparations with NPY (10 nM) significantly attenuated SP-mediated secretion, application of 1 μM NPY (13–36) did not significantly reduce SP responses. Given this additional statistical information, the reduced spectrum of activity observed with these and presumably the other fragments may indicate the presence of prejunctional NPY receptors with $Y_2$-like characteristics.

According to the classification of Wahlestedt *et al.* (1986) there are two types of NPY receptors at sympathetic neuroeffector junctions; $Y_1$-receptors requiring the full length of NPY and $Y_2$-receptors which recognise truncated forms of NPY. The former appeared to be postjunctional, while the latter were found to be prejunctional in these vascular tissues. Whilst this subdivision of NPY receptors is becoming widely accepted, the preferential location of $Y_1$ and $Y_2$ to post- and prejunctional membranes respectively does not necessarily follow. Both types of NPY receptors have been observed in a range of human neuroblastoma cell lines and the pheochromocytoma cell line, PC12 (Sheikh *et al.*, 1989a,b). $Y_2$-like receptors have been identified in postjunctional membranes such as those of pig spleen (Lundberg *et al.*, 1988) and rabbit proximal tubule epithelia (Sheikh *et al.*, 1989c).

The antisecretory effects of NPY in rat jejunum would appear to be mediated by $Y_2$-receptors given that NPY (11–36), (12–36), (13–36) and (14–36) and shorter fragments will, albeit at high concentrations, produce direct reductions in basal s.c.c. From all the foregoing data it was found that by expressing the $EC_{50}$ for NPY (11–36) and NPY (13–36) with respect to NPY, ratios of 13.0 and 67.0 were obtained respectively for EFS responses, while ratios of 154.0 for both in respect of basal current were obtained. This together with the potency order NPY $\gg$ NPY (11–36) = NPY (13–36) for basal and NPY > NPY (11–36) > NPY (13–36) for EFS, indicate subtle differences between pre- and postjunctional actions of NPY and its fragments. The classification of subdivisions of receptors with agonists is notoriously difficult (especially as some of the less potent, shorter fragments may be partial agonists), and the possibility of a combination of $Y_1$ and $Y_2$-like receptors existing on both surfaces cannot be ruled out at this stage. Only when selective agonists (Fuhlendorff *et al.*, 1990) and, more importantly, NPY antagonists become available will the subclassification of these receptors be clarified. For the moment NPY receptors with $Y_2$-like properties appear to be present within prejunctional, submucous neurones and postjunctional epithelial membranes in the rat small intestine.

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## References

COOKE, H.J. (1984). Influence of enteric cholinergic neurons on mucosal transport in guinea-pig ileum. *Am. J. Physiol.*, **246**, G263–G267.

COOKE, H.J., SHONNARD, K. & WOOD, J.D. (1983). Effect of neuronal stimulation on mucosal transport in guinea-pig ileum. *Am. J. Physiol.*, **245**, G290–G295.

COX, H.M. & CUTHBERT, A.W. (1988). NPY antagonises secretagogue evoked chloride transport in rat jejunal epithelium. *Pflügers Arch.*, **413**, 38–42.

COX, H.M., CUTHBERT, A.W., HÅKANSON, R. & WAHLESTEDT, C. (1988). The effect of NPY and PYY on electrogenic ion transport in rat intestinal epithelia. *J. Physiol.*, **398**, 65–80.

EKBLAD, E., HÅKANSON, R. & SUNDLER, F. (1984). VIP and PHI coexist with a NPY-like peptide in intramural neurones of the small intestine. *Reg. Peptides*, **10**, 47–55.

EKBLAD, E., WINTHER, C., EKMAN, R., HÅKANSON, R. & SUNDLER, F. (1987). Projections of peptide-containing neurones in rat small intestine. *Neuroscience*, **20**, 169–188.

FURNESS, J.B., COSTA, M., GIBBINS, I.L., LLEWELLYN-SMITH, I.J. & OLIVER, J.R. (1985). Neurochemically similar myenteric and submucous neurones directly traced to the mucosa of the small intestine. *Cell Tissue Res.*, **241**, 155–163.

FUHLENDORFF, J., GETHER, U., AAKERLUND, L., LANGELAND-JOHANSEN, N., THØGERSEN, H., MELBERG, S.G., BANG OLSEN,

U., THASTRUP, O. & SCHWARTZ, T.W. (1990). [Leu$^{31}$, Pro$^{34}$] Neuropeptide Y: a specific $Y_1$ receptor agonist. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 182–186.

GAGINELLA, T.S., O'DORISIO, T.M. & HUBEL, K.A. (1981). Release of vasoactive intestinal peptide by electric field stimulation of rabbit ileum. *Reg. Peptides*, **2**, 165–174.

HOLZER, P., LIPPE, I.TH., BARTHO, L. & SARIA, A. (1987). NPY inhibits excitatory enteric neurons supplying the circular muscle of the guinea pig small intestine. *Gastroenterology*, **92**, 1944–1950.

HUBEL, K.A. (1978). The effects of electric field stimulation and tetrodotoxin on ion transport by the isolated rabbit ileum. *J. Clin. Invest.*, **62**, 1039–1047.

HUBEL, K.A. (1984). Electrical stimulus-secretion coupling in rabbit ileal mucosa. *J. Pharmacol. Exp. Ther.*, **231**, 577–582.

KEAST, J.R., FURNESS, J.B. & COSTA, M. (1985). Investigations of nerve populations influencing ion transport that can be stimulated electrically, by serotonin and by a nicotinic agonist. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **331**, 260–266.

KENAKIN, T.P. (1983). Receptor classification by selective agonists: coping with circulatory and circumstantial evidence. *Trends Pharmacol. Sci.*, **4**, 291–295.

KRSTENANSKY, J.L. & BUCK, S.H. (1987). The synthesis, physical characterisation and receptor binding affinity of NPY. *Neuropeptides*, **10**, 77–85.

KRSTENANSKY, J.L., OWEN, T.J., BUCK, S.H., HAGAMAN, K.A. & McLEAN, L.R. (1989). Centrally truncated and stabilised porcine neuropeptide Y analogs: design, synthesis and mouse brain receptor binding. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 4377–4381.

LEBLANC, G.G., TRIMMER, B.A. & LANDIS, S.C. (1987). NPY-like immunoreactivity in rat cranial parasympathetic neurons: coexistence with VIP and choline acetyl transferase. *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 3511–3515.

LUNDBERG, J.M., HEMSEN, A., LARSSON, O., RUDEHILL, A., SARIA, A. & FREDHOLM, B.B. (1988). NPY receptor in pig spleen; binding characteristics, reduction of cAMP formation and calcium antagonist inhibition of vasoconstriction. *Eur. J. Pharmacol.*, **145**, 21–29.

MacKERELL, A.D., HEMSEN, A., LACROIX, J.S. & LUNDBERG, J.M. (1989). Analysis of structure-function relationships and neuropeptide Y using molecular dynamics simulations and pharmacological activity and binding measurements. *Reg. Peptides*, **25**, 295–313.

McCULLOCH, C.R., KUWAHARA, A., CONDON, C.D. & COOKE, H.J. (1987). Neuropeptide modification of chloride secretion in guinea pig distal colon. *Reg. Peptides*, **19**, 35–43.

POTTER, E.K., MITCHELL, L., McCLOSKEY, M.J.D., TSENG, A., GOODMAN, A.E., SHINE, J. & McCLOSKEY, D.I. (1989). Pre- and postjunctional actions of NPY and related peptides. *Reg. Peptides*, **25**, 167–171.

RIOUX, F., BACHELARD, H., MARTEL, J.C. & ST PIERRE, S. (1986). The vasoconstrictor effect of NPY and related peptides in the guinea pig isolated heart. *Peptides*, **7**, 27–31.

SERVIN, A.L., ROUYER-FESSARD, C., BALASUBRAMANIAM, A., SAINT PIERRE, S. & LABURTHE, M. (1989). Peptide YY and neuropeptide Y inhibit VIP-stimulated cAMP production in rat small intestine: structural requirements of peptides for interacting with peptide YY preferring receptors. *Endocrinology*, **124**, 692–700.

SHEIKH, S.P., HÅKANSON, R. & SCHWARTZ, T.W. (1989a). $Y_1$ and $Y_2$ receptors for neuropeptide Y. *FEBS Lett.*, **245**, 209–214.

SHEIKH, S.P., O'HARE, M.M.T., TORTORA, O. & SCHWARTZ, T.W. (1989b). Binding of monoiodinated neuropeptide Y to hippocampal membranes and human neuroblastoma cell lines. *J. Biol. Chem.*, **264**, 6648–6654.

SHEIKH, S.P., SHEIKH, M.I. & SCHWARTZ, T.W. (1989c). $Y_2$-type receptors for peptide YY on renal proximal tubular cells in the rabbit. *Am. J. Physiol.*, **257**, F978–F984.

SUNDLER, F., MOGHIMZADEH, E., HÅKANSON, R., EKELUND, M. & EMSON, P.C. (1983). Nerve fibres in the gut and pancreas of the rat displaying NPY immunoreactivity. *Cell Tissue Res.*, **230**, 487–493.

WAHLESTEDT, C., YANAIHARA, N. & HÅKANSON, R. (1986). Evidence for different pre- and postjunctional receptors for NPY and related peptides. *Reg. Peptides*, **13**, 307–318.

WAHLESTEDT, C., EDVINSSON, L., EKBLAD, E. & HÅKANSON, R. (1987). Effects of NPY at sympathetic neuroeffector junctions: existence of $Y_1$ and $Y_2$ receptors. In *Neuronal Messengers in Vascular Function*, ed. Arnedo-Nobin, B., Nobin, A. & Owman, C.L. (Fernström) Symposia No 8, pp. 231–242. Amsterdam: Elsevier.

WATTCHOW, D.A., FURNESS, J.B., COSTA, M., O'BRIEN, P.E. & PEACOCK, M. (1987). Distribution of neuropeptides in the human esophagus. *Gastroenterology*, **93**, 1363–1371.

WILEY, J. & OWYANG, C. (1987). NPY inhibits cholinergic transmission in the isolated guinea pig colon: mediation through α-adrenergic receptors. *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 2047–2051.

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