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# Presynaptic inhibition by neuropeptide Y in rat hippocampal slice *in vitro* is mediated by a $Y_2$ receptor

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1 The action of analogues and C-terminal fragments of neuropeptide Y (NPY) was examined on excitatory synaptic transmission in area CA1 of the rat hippocampal slice *in vitro*, by use of intracellular and extracellular recordings, to determine by agonist profile the NPY receptor subtype mediating presynaptic inhibition.

2 Neither NPY, analogues nor fragments of NPY affected the passive or active properties of the postsynaptic CA1 pyramidal neurones, indicating their action is at a presynaptic site.

3 The full-sequence analogues, peptide YY (PYY) and human NPY (hNPY), were equipotent with NPY at the presynaptic receptor, while desamido hNPY was without activity.

4 NPY2–36 was equipotent with NPY. Fragments as short as NPY13–36 were active, but gradually lost activity with decreasing length. NPY16–36 had no effect on extracellular field potentials, but still significantly inhibited excitatory postsynaptic potential amplitudes. Fragments shorter than NPY16–36 had no measurable effect on synaptic transmission.

5 The presynaptic NPY receptor in hippocampal CA1 therefore shares an identical agonist profile with the presynaptic $Y_2$ receptor at the peripheral sympathetic neuroeffector junction.

## Introduction

Neuropeptide Y (NPY) is an abundant, 36 amino acid peptide expressed and released by many types of nerve cells in the central (CNS) and peripheral (PNS) nervous systems of mammals (DeQuidt & Emson, 1986; Sundler *et al.*, 1986). In peripheral smooth muscle tissues, NPY has two sites of action: (1) on the muscle cell itself, relatively high concentrations of NPY induce contractions, and subthreshold concentrations of the peptide markedly enhance the contractile response to other agents, such as noradrenaline and histamine (Wahlestedt *et al.*, 1986; Wahlestedt, 1987); (2) on the presynaptic sympathetic nerve terminal, NPY inhibits its own release, as well as the release of noradrenaline (Wahlestedt *et al.*, 1986; Wahlestedt, 1987). In rat hippocampal slice *in vitro*, NPY has been shown to inhibit excitatory synaptic transmission at stratum radiatum-CA1 (glutamatergic) synapses (Colmers *et al.*, 1985; 1987; 1988; Haas *et al.*, 1987) by an action at the presynaptic terminals, probably the inhibition of voltage-dependent calcium influx (Colmers *et al.*, 1988).

To date, no antagonists at NPY receptors have been identified. However, studies which tested agonist fragments and analogues of NPY indicate that at least two subtypes of NPY receptor can be distinguished pharmacologically in peripheral sympathetic neuroeffector junctions (Wahlestedt *et al.*, 1986; Wahlestedt, 1987). The $Y_1$ subtype is located on the postsynaptic cell in blood vessels and vas deferens, and requires the intact NPY, or the very closely related peptide YY (PYY) molecule for its activation (Wahlestedt *et al.*, 1986). The $Y_2$ subtype is found on the presynaptic terminal, and can be activated by analogues and C-terminal fragments of NPY as short as NPY 13–36 (Wahlstedt *et al.*, 1986). The C-terminal desamido form of NPY has very little activity at both receptor subtypes (Wahlestedt *et al.*, 1986). The two receptor subtypes in peripheral tissues are also distinguished by the second messenger systems to which they are coupled; $Y_1$ receptor activation causes phosphatidylinositol hydrolysis, while $Y_2$ receptor activation inhibits adenylate cyclase (Wahlestedt, 1987; Westlind-Danielsson *et al.*, 1987; Hinson *et al.*, 1988; Perney & Miller, 1989).

To ascertain which NPY receptor subtype (if either) is responsible for the presynaptic inhibition observed in hippocampus, we compared the response of stratum radiatum-CA1 excitatory synaptic transmission to single concentrations of agonist analogues and fragments of NPY with that to the intact peptide. The results indicate that, in rat hippocampus *in vitro*, NPY inhibits excitatory synaptic transmission via a receptor which is pharmacologically similar in its agonist profile to the peripheral $Y_2$ subtype.

## Methods

### *Peptide synthesis*

Fragments of NPY were synthesized by the solid phase method with a manual home-made multireactor synthesizer. The syntheses were carried out with a benzhydrylamine resin (Pietta *et al.*, 1974) since the peptides bear an amide C-terminal function. All amino acids were coupled via the BOP/DMF method (Fournier *et al.*, 1988), according to a recently-described protocol (Forest & Fournier, 1989). The Boc amino acids with appropriate side-chain protection were obtained from commercial sources. Completed peptides were cleaved from the resin support and deprotected by a 90 min treatment at 0°C with liquid hydrofluoric acid containing *m*-cresol and dimethyl sulphide as scavengers (10:1:1 v/v).

After extraction from the resin and lyophilization, the peptides were purified by reverse-phase chromatography on a Waters Deltapak column, using an eluent of (A) $H_2O$ (0.06% trifluoroacetic acid, TFA) and (B) acetonitrile-$H_2O$ (0.06% TFA). Peptides were eluted with successive linear gradients of solvent B. Analytical high performance liquid chromatography (h.p.l.c.) of the individual fractions was carried out and the fractions corresponding to the purified peptide were lyophilized. The purified material was characterized by analytical h.p.l.c., capillary electrophoresis and amino acid analysis.

### *Electrophysiological studies*

Intra- and extracellular recordings were made from area CA1 of rat hippocampal slices as described previously by Colmers

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et al. (1987, 1988). Briefly, transverse slices (400–450 $\mu$m) of hippocampus were submerged in a recording chamber continuously perfused (2.5 ml min$^{-1}$) with saline (Colmers et al., 1985; 1987; 1988) saturated with 95% $O_2$/5% $CO_2$ and heated to 34 ± 0.2°C. Orthodromic stimuli (monophasic, square wave, 100–300 $\mu$s, 3–35 V) were applied through bipolar electrodes, placed on stratum radiatum of area CA1. Extracellular recordings were made from the stratum pyramidale of area CA1 with glass microelectrodes (3–15 MΩ; 2 M NaCl); intracellular recordings were made from CA1 pyramidal neurones in area CA1 with glass microelectrodes (85–150 MΩ; 2 M $K^+$ acetate). An Axoclamp 2A amplifier, used in the bridge current clamp mode was used both for extracellular and intracellular recordings. Current and voltage were displayed continuously on a d.c. coupled chart recorder (Gould 2200, frequency response d.c. 60 Hz), and selected parts of experiments were stored either on FM (Racal) or PCM coded videotape (Vetter). A Nicolet 4094 digital oscilloscope was used to average and store data on-line and analyse data off-line.

Analysis of electrophysiological data was performed as published previously (Colmers et al. 1985; 1987; 1988). Stimulus amplitudes were chosen to evoke responses between 70 and 85% of maximum, which is on the steepest, and therefore most sensitive, portion of the stimulus-response relationship (Colmers et al., 1985; 1987). Population spike (PS) amplitude was determined from the peak of the negativity following the stimulus artifact to the peak of the following positivity. Resting membrane potential was determined from chart records; excitatory postsynaptic potentials (e.p.s.ps) were measured as peak amplitudes. E.p.s.ps were evoked 40 ms after beginning of a hyperpolarizing current pulse applied to the electrode via the bridge circuit to prevent the neurone from achieving action potential threshold. Input resistance was determined by the slope of a least-squares linear regression line fitted to data obtained from families of hyperpolarizing and depolarizing constant current pulses (125 ms) applied to neurones under each condition.

Peptides and drugs were applied via the superfusate (Colmers et al., 1987; 1988). Native porcine NPY (Richelieu Biotechnologies, Québec, Canada or a generous gift from Dr T.O. Neild, Monash University, Melbourne, Australia) and analogues and fragments (synthesized as above) were prepared just prior to use at a final concentration of 1 $\mu$M. All analogues and fragments, except as noted, were porcine NPY sequence, C-terminally amidated peptides. Native, intact porcine NPY is referred to here simply as NPY. Fragments are referred to by the number of C-terminal residues.

Data were taken only from preparations where a significant recovery from drug effects occurred upon washout. Preparations were used as their own controls for statistical purposes, and peptide effects relative to control were assessed by Student's paired $t$ test. Although we attempted to apply NPY itself to each preparation, it was not always possible; pooled data from fragments and analogues were therefore compared statistically with one another by use of Student's $t$ test for 2 means.

## Results

Results are based on recordings from 96 separate preparations: extracellular recordings were made in 83 different preparations; intracellular recordings were made of 62 different neurones in some of the preparations where field potentials were simultaneously recorded, and in 10 preparations without simultaneous extracellular recordings.

As we and others have observed previously (Colmers et al., 1985; 1987; 1988; Haas et al., 1987), NPY reversibly inhibited excitatory synaptic transmission from stratum radiatum to CA1 pyramidal cells, as measured by a reduction of the amplitude of the extracellular population spike (PS). A concentration-response relationship to NPY indicated the threshold for inhibition of the PS to be about 30 nM; 1 $\mu$M inhibited the PS by 89.9 ± 2.8% ($n$ = 16). The $EC_{50}$ for NPY on the PS was about 250 nM, similar to that seen in earlier studies (Colmers et al., 1985; 1987).

In addition, as reported earlier (Colmers et al., 1987; 1988; Haas et al., 1987), NPY also reversibly inhibited the intracellularly-recorded e.p.s.p. evoked in CA1 pyramidal cells by stratum radiatum stimulation. NPY, 1 $\mu$M, reduced the e.p.s.p. by 59.8 ± 2.3% ($n$ = 8); the $EC_{50}$ for NPY effects on e.p.s.p. was about 200 nM, in agreement with earlier findings (Colmers et al., 1987). As reported earlier, NPY had no effects on passive or active membrane properties in CA1 neurones (Colmers et al., 1987; 1988).

### *Peptide analogues*

All peptide analogues and fragments tested in this study were without effects on membrane resting potential, input resistance or action potential amplitude or duration, consistent with earlier observations on NPY itself (Colmers et al., 1987; 1988).

Application of the full-sequence native analogue, PYY, at 1 $\mu$M also caused reduction of both PS and e.p.s.p. amplitudes, which reversed upon washout (Figure 1). The inhibition of PS was statistically indistinguishable from that seen with NPY (Figure 2) although it appeared greater and persisted longer. However, the effect of PYY on the simultaneously-recorded intracellular e.p.s.p. was indistinguishable from that of NPY (Figure 3) although the effect also appeared to persist longer than with NPY.

Human sequence NPY (hNPY), the amino acid sequence of which is identical with rat NPY and differs from that of porcine NPY only at position 17 (Corder et al., 1984; Minth et al., 1984; Allen et al., 1987), was as effective as NPY in inhibiting synaptic transmission measured both as PS (Figures 1 and 2) and e.p.s.p. (Figures 1 and 3). However, desamido hNPY (hNPYa) had no effects on synaptic transmission (Figure 2).

### *Peptide fragments*

Amidated C-terminal fragments of (porcine sequence) NPY, including NPY2–36, 5–36, 11–36, 16–36, and 25–36, were also studied. Data are summarized in Figures 2, 3 and 4.

NPY2–36 was at least as potent as NPY in inhibiting excitatory synaptic transmission in CA1. No significant difference

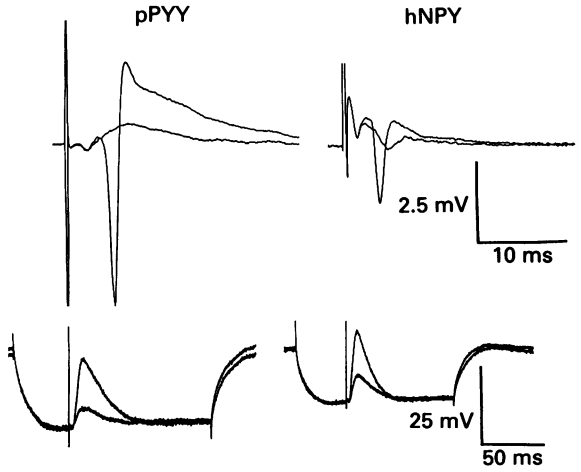


**Figure 1** Effect of the full-sequence neuropeptide Y (NPY) analogues, peptide YY (PYY, left traces) and human NPY (hNPY, right traces), on amplitude of the population spike (PS, upper traces) and excitatory postsynaptic potential (e.p.s.p., lower traces) recorded simultaneously in CA1. Records for PYY and hNPY are from different preparations. Peptides were applied at a concentration of 1 $\mu$M. Control and peptide traces are shown superimposed for comparison. The responses recovered after prolonged washout (not illustrated).

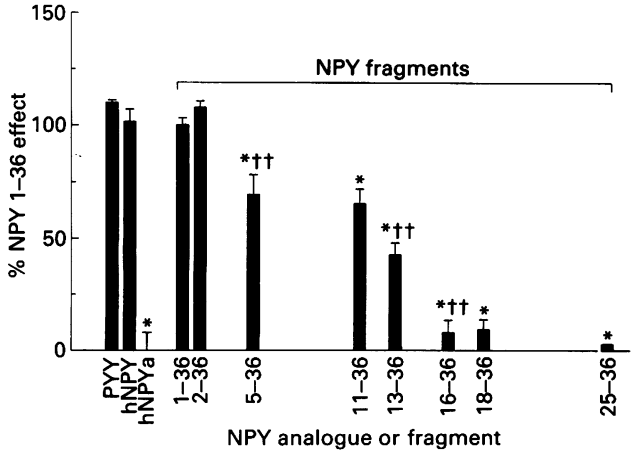


**Figure 2** Relative effects of analogues and C-terminal fragments of neuropeptide Y (NPY) on population spike (PS) evoked in CA1 by stimulation of stratum radiatum. Values shown are means of effects (s.e.mean shown by vertical bars), expressed as a percentage of mean NPY (=100%) effect. Analogues are shown on the left of the figure, while pNPY and its fragments are shown to the right (under bar), listed in order of decreasing length on the X-axis, with spacing along this axis indicating the relative length of the fragments. * $P < 0.001$ difference from NPY; † $P < 0.02$ difference from previous fragment; †† $P < 0.001$ difference from previous fragment. All fragments were tested ≥5 times in different preparations. Fragments NPY16–36 and shorter were without significant effect at inhibiting the PS.

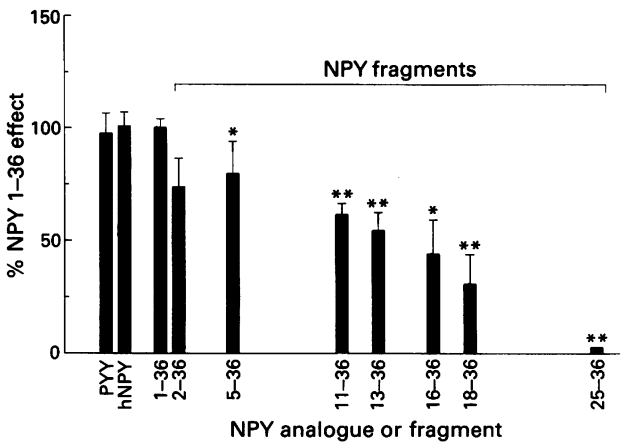


**Figure 3** Relative effects of analogues and C-terminal fragments of neuropeptide Y (NPY) on excitatory postsynaptic potential (e.p.s.p.) evoked in CA1 pyramidal neurones by stimulation of stratum radiatum. Values shown are means of effects (s.e.mean shown by vertical bars), expressed as a percentage of mean NPY (=100%) effect. Analogues and fragments are distributed as in Figure 2. * $P < 0.005$ difference from NPY; ** $P < 0.001$ difference from NPY. Immediately neighbouring fragments were not statistically different from one another. All fragments were tested ≥5 times on different preparations. Fragments NPY18–36 and shorter were without effect on the e.p.s.p.

was seen between this fragment's actions on both PS and e.p.s.p. and those of NPY. While NPY5–36 also inhibited excitatory synaptic transmission, it was less active than NPY. At a concentration of 1 $\mu$M, this fragment was about 70% as active in inhibiting the PS and about 80% as active in inhibiting the e.p.s.p. as was NPY. NPY5–36 was also significantly less active than NPY2–36, NPY11–36 was slightly, but not significantly, less active at the presynaptic NPY receptor than was NPY5–36. However, NPY13–36 was significantly less active than NPY11–36, and was less than half as active as the intact peptide in inhibiting the PS, while its inhibition of the e.p.s.p. was somewhat more than 50% of that caused by NPY itself (Figure 2). By contrast, the slightly shorter NPY16–36 fragment did not significantly inhibit PS in CA1, although it had a small, but significant, inhibitory effect on the e.p.s.p. recorded intracellularly. NPY16–32 was, on average, 80% as active as NPY13–36 in inhibiting e.p.s.p. amplitudes. NPY18–36 was without any significant action on either the PS or the e.p.s.p., and NPY25–36 was without significant effects at 1 $\mu$M on PS or e.p.s.p. (Figure 3).

## Discussion

In this study, there were no effects of NPY, its analogues or fragments on the passive or active membrane properties of the CA1 pyramidal neurones. This is in agreement with other work from this laboratory (Colmers *et al.*, 1987; 1988) and others (Haas *et al.*, 1987). Thus, all active peptides tested on stratum radiatum-CA1 synaptic transmission *in vitro* appear to act only at a presynaptic site.

The presynaptic receptor for NPY in the rat hippocampus appears to share an identical agonist profile with the $Y_2$ receptor first characterized at sympathetic neuroeffector junctions (Wahlestedt *et al.*, 1986; Wahlestedt, 1987). Thus, the full-sequence analogues PYY and hNPY are equipotent with NPY, while desamido hNPY was without measurable activity, confirming previous reports that the C-terminal amide is necessary for any activity of the peptide at either $Y_1$ or $Y_2$ receptors (Wahlestedt *et al.*, 1986). The 2–36 fragment of NPY was at least equipotent with NPY, although further reductions in fragment length caused a gradual decline in activity. There was a relatively sharp drop in activity between NPY13–36, which had roughly 50% of the activity of NPY, and NPY16–

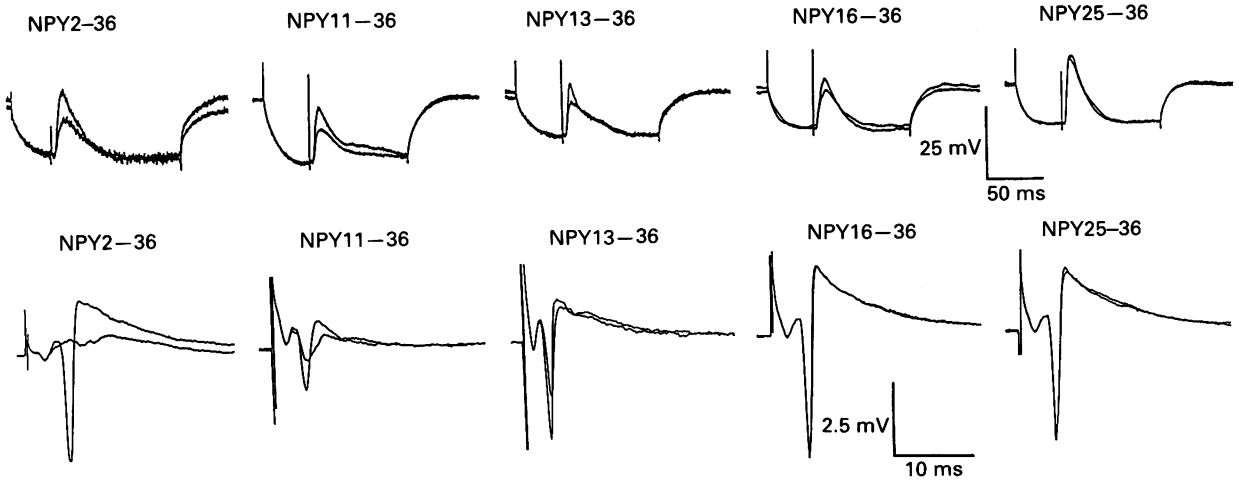


**Figure 4** Representative effects of different C-terminal fragments of neuropeptide Y (NPY) on excitatory postsynaptic potential (e.p.s.p., upper traces) and population spike (PS, lower traces) applied at 1 $\mu$M in CA1. Fragment length is indicated above each trace. Records are all from different preparations. All responses showed recovery upon washout (not illustrated).

36, which had no measurable effect on the PS, and a small, but significant effect on the e.p.s.p. Further truncation to NPY18–36 eliminated all activity at the presynaptic receptor in hippocampus.

While NPY16–36 had a small but significant effect on e.p.s.ps, it did not affect PS in this study. $Y_2$ receptors in peripheral tissue have been shown to be sensitive to fragments as short as NPY23–36 (Grundemar & Håkanson, 1990); NPY18–36 inhibits calcium influx in dorsal root ganglion cells in culture (Colmers, Bleakman & Miller, unpublished). It therefore seems likely that, at the concentrations tested here, the actions of NPY16–36 were subthreshold for an effect on PS. It is therefore essential that both pre- and postsynaptic responses be examined in similar studies.

The $Y_2$ receptor has been shown in peripheral tissues and in homogenates of whole brain to inhibit the activity of adenylate cyclase (Wahlestedt, 1987; Westlind-Danielsson *et al.*, 1987). However, although the mechanism coupling the presynaptic NPY receptor in hippocampus to its effectors (probably calcium channels in presynaptic terminals; Colmers *et al.*, 1988) is not known, preliminary results indicate that the inhibition of adenylate cyclase is not responsible, as elevation of intracellular adenosine 3':5'-cyclic monophosphate levels with a membrane-soluble analogue did not affect the inhibition of synaptic transmission brought about by NPY (Klapstein *et al.*, 1990). The actual mechanism by which NPY exerts its presynaptic action is currently under investigation.

As the results of this and other studies indicate, some presynaptic nerve terminals in both the peripheral and central nervous systems bear NPY receptors which inhibit release of transmitter. Thus far, they all appear to be of the $Y_2$ subtype. The notable difference is that, in peripheral nerve terminals, the NPY receptor is an inhibitory autoreceptor, while in hippocampus, it is an inhibitory heteroreceptor. While the inhibition of synaptic transmission by action at a presynaptic terminal appears a common property of the $Y_2$ receptors studied until now, there seems to be a difference in the role they play in different neuronal systems.

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