J. Physiol. (1987), **383**, *pp.* 285–299
With 8 text-figures
Printed in Great Britain

PRESYNAPTIC ACTION OF NEUROPEPTIDE Y IN AREA CA1 OF THE RAT HIPPOCAMPAL SLICE

BY WILLIAM F. COLMERS, KEN LUKOWIAK AND QUENTIN J. PITTMAN

From the Department of Medical Physiology, University of Calgary, Calgary, Alberta T2N 4N1, Canada

(*Received 23 October 1985*)

SUMMARY

1. Neuropeptide tyrosine (neuropeptide Y, NPY), a recently isolated endogenous brain peptide, reduces the extracellular population spike evoked by stimulation of stratum radiatum in area CA1 of the *in vitro* rat hippocampal slice, without reducing the antidromically evoked population spike. To test the hypothesis that NPY acts presynaptically, intracellular recordings were made of pyramidal neurones of area CA1 *in vitro*.

2. Bath application of 10^{-6} M-NPY causes a long-lasting (1–1·5 h), reversible reduction of the orthodromically evoked excitatory post-synaptic potential (e.p.s.p.) recorded intracellularly from CA1 pyramidal neurones. This effect on the e.p.s.p. was dependent upon the concentration of NPY.

3. The resting membrane potential, slope input resistance, and action potential threshold, amplitude and duration of the CA1 pyramidal neurones were not affected by NPY.

4. The responses of CA1 pyramidal neurones to ionophoretic pulses of glutamate, applied to the dendrites during synaptic blockade, was also unaffected by NPY.

5. The evidence supports the hypothesis that NPY acts presynaptically in the CA1 region of hippocampus to reduce excitatory input to the pyramidal neurones.

INTRODUCTION

Neuropeptide tyrosine (neuropeptide Y, NPY), a thirty-six-amino-acid member of the pancreatic polypeptide family, was first isolated from porcine brain in 1982 by Tatemoto. NPY has subsequently been localized, using a variety of antibodies, to many central nervous system (C.N.S.) structures (Adrian, Allen, Bloom, Ghatei, Rossor, Roberts, Crow, Tatemoto & Polak, 1983; Allen, Adrian, Allen, Tatemoto, Crow, Bloom & Polar, 1983; Everitt, Hokfelt, Terenius, Tatemoto, Mutt & Goldstein, 1984; Chronwall, DiMaggio, Massari, Pickel, Ruggiero & O'Donohue, 1985), and to the peripheral nervous system (P.N.S.) and intrinsic innervation of most organs, including gut, heart and the vasculature (Edvinsson, Emson, McCulloch, Tatemoto & Uddman, 1983; Lundberg, Terenius, Hokfelt & Goldstein, 1983; Sundler, Moghimzadeh, Håkanson, Ekelund & Emson, 1983; Gu, Polak, Allen, Huang, Sheppard, Tatemoto & Bloom, 1984). NPY is one of the most abundant peptides in the nervous system of mammals (Allen *et al.* 1983; Chronwall *et al.* 1985).

In addition to its abundance, NPY is of interest because of its coexistence with other classical neurotransmitters. In the P.N.S., NPY has been co-localized with noradrenaline (NA) and adrenaline (Lundberg, Terenius, Hokfelt, Martling, Tatemoto, Mutt, Polak, Bloom & Goldstein, 1982; Lundberg *et al.* 1983; Ekblad, Edvinsson, Wahlestedt, Uddman, Håkanson & Sundler, 1984). In the C.N.S., NPY-like immunoreactivity has been demonstrated in the same brain-stem neurones as NA and adrenaline (Everitt *et al.* 1984), and in neurones throughout the neocortex which contain γ-amino butyric acid (GABA), (Hendry, Jones, DeFelipe, Schmechel, Brandon & Emson, 1984).

Physiological evidence to date indicates that NPY acts at several loci. In vascular tissue, it has a direct vasoconstrictor effect, and enhances the action of other vasoconstrictor agents such as NA, adrenaline and histamine (Lundberg *et al.* 1982; Lundberg & Tatemoto, 1982; Edvinsson, Ekblad, Håkanson & Wahlestedt, 1984; Ekblad *et al.* 1984). By contrast, NPY has been shown to inhibit the electrically stimulated contractions of vas deferens and urinary bladder, without affecting their responses to exogenously applied transmitter substances, leading several investigators to conclude a presynaptic action of the peptide (Ohhashi & Jacobowitz, 1983; Lundberg, Hua & Franco-Cereceda, 1984; Allen, Adrian, Tatemoto, Polak, Hughes & Bloom, 1982). In vas deferens, NPY has been shown to inhibit the electrically stimulated release of NA, also via a presynaptic mechanism (Lundberg & Stjarne, 1984).

In the C.N.S., exogenously applied NPY has physiological effects which appear to be independent of the action of NA. Injection of NPY intracerebroventricularly in anaesthetized rats causes bradypnoea, hypotension, and e.e.g. synchronization (Fuxe, Agnati, Harfstrand, Zini, Tatemoto, Pich, Hokfelt, Mutt & Terenius, 1983) and modulates release of gonadotropin (Kalra & Crowley, 1984). Micro-injections of picomolar to nanomolar quantities of NPY into the paraventricular nucleus (p.v.n.) of the hypothalamus in awake rats causes an increase in feeding (Stanley & Leibowitz, 1985). Injection of NPY into the supraoptic nucleus affects levels of vasopressin in the circulation (Willoughby & Blessing, 1985). In the hamster, micro-injection of NPY into the suprachiasmatic nucleus (s.c.n.) of the hypothalamus causes a phase shift in circadian rhythms (Albers & Ferris, 1984).

NPY is present in relatively large amounts in rat hippocampus (Chronwall *et al.* 1985), and exogenous application to this structure *in vitro* has been shown to reduce the orthodromic population spike (p.s.) of the extracellular field potential evoked in area CA1 by electrical stimulation of the stratum radiatum (s.r.) (Colmers, Lukowiak & Pittman, 1985). The antidromic population spike, evoked in area CA1 by stimulation from the alveus, was unaffected by NPY. This raised the possibility that NPY acts presynaptically in the SR–CA1 pathway to reduce excitatory input to the CA1 pyramidal neurones. To test this hypothesis, we studied the effect of NPY on the synaptic and post-synaptic properties of CA1 pyramidal neurones *in vitro*.

METHODS

Transverse slices of hippocampus, 350 μm thick, were taken from 75–150 g male Sprague-Dawley rats and prepared as described previously (Pittman & Siggins, 1981). Slices were incubated, submerged, on nylon mesh in a Plexiglass recording chamber at $32 \pm 0{\cdot}2$ °C. They were perfused

at a constant rate of 2–3 ml/min with an artificial cerebrospinal fluid (ACSF) containing (mM): NaCl, 124; $NaHCO_3$, 26; glucose, 10; $MgSO_4$, 2; KCl, 1·8; $CaCl_2$, 1·5; and KH_2PO_4, 1·25. For synaptic blockade the medium was identical, except that $CaCl_2$ was 0·5 mM, and $MgCl_2$ was 3·5 mM. The ACSF was bubbled throughout with a gas mixture of 95 % O_2, 5 % CO_2, and had a pH of 7·40.

Electrophysiological recordings

An electrode containing 2–3 M-NaCl, having a resistance of 2–6 MΩ and connected to a Getting model 5 amplifier, was placed near the stratum pyramidale of area CA1 to measure the extracellular field potential. A bipolar stimulating electrode of nichrome wire was placed in the s.r. to activate afferent fibres to CA1. The s.r. was stimulated at 0·1 Hz, using a single, square-wave, monophasic pulse (100 μs, 2–20 V) from a stimulus isolation unit (Digitimer DS2). The population spike amplitude was measured from the peak of the negative wave to the peak of the following positive one (Colmers *et al.* 1985). In some experiments, a second extracellular electrode was placed in the region of the apical dendrites of the CA1 pyramidal neurones, in order to record simultaneously the field excitatory post-synaptic potential (e.p.s.p.) evoked by a s.r. stimulus (Andersen, Sundberg, Sveen, Swann & Wigstrom, 1980). Once the population spike amplitude has been stable for at least 45 min, an electrode filled with 2 M-potassium acetate and having a resistance of 100–220 MΩ, connected via a chlorided silver wire to a Dagan Model 8100 amplifier, was lowered into the cell body layer of area CA1 near the location of the extracellular electrode. Pyramidal neurones were impaled and identified using standard electrophysiological criteria, including action potential shape and amplitude, as well as a response to s.r. stimulation (Andersen *et al.* 1980). Neurones were selected whose resting potential was stable at more than 50 mV for more than 20 min. These neurones sometimes received spontaneous e.p.s.p.s, but were prevented from reaching threshold by injection of constant amounts of hyperpolarizing current through the bridge circuit of the amplifier, whose balance was carefully monitored. Constant-current pulses injected via the bridge circuit through the electrode were used for the estimation of input resistance.

The e.p.s.p. was evoked during a pulse of hyperpolarizing current (150–200 ms long) to prevent spiking (Andersen *et al.* 1980). The s.r. was stimulated only after the membrane potential had stabilized to this pulse. The e.p.s.p. amplitude was measured from the membrane potential after the shock artifact had completely subsided to the peak amplitude of the e.p.s.p. The stimulating voltage was varied from near threshold for the e.p.s.p. to the maximum amplitude possible without the neurone reaching action potential threshold. Once the relationships between stimulus voltage and both population spike and e.p.s.p. amplitudes had been established, synthetic porcine NPY (Bachem Inc., Torrance, CA, U.S.A.), previously dissolved in ACSF at a concentration of 10^{-4} M and stored in aliquots at −80 °C until just prior to use, was diluted to the desired concentration. 10 ml peptide solution was applied through the bath via a switching valve; care was taken to ensure a constant rate of flow through the bath during the drug runs. The drug was washed out with ACSF.

Data were collected on a d.c.-coupled pen recorder (Gould), which continuously recorded cell membrane potential and injected current throughout the duration of the experiment. In addition, a digital storage oscilloscope (Nicolet model 4094) was used to observe and record individual population spikes, e.p.s.p.s and the current pulses used to either depolarize or hyperpolarize the neurone on floppy diskettes. This instrument was also used to average signals either on-line or from tape. Critical parts of the experiments, including the control and drug runs, were recorded on FM tape (Racal Store 4DS) for subsequent analysis.

Ionophoresis experiments

For experiments involving the ionophoretic application of glutamate to the dendrites of an impaled neurone, a glass micropipette, filled with 0·25 M-sodium glutamate at pH 8·0, resistance 4–10 MΩ, was placed in the region of the apical dendrites of the CA1 pyramidal neurones. A Neurophore BH2 mainframe with an IP2 ionophoresis pump (Medical Systems, Great Neck, NY, U.S.A.) was used to pass 100–300 ms, 350–500 nA pulses of current at 0·05 Hz to eject glutamate from the electrode tip. A backing current of 10 nA was used to prevent passive diffusion of glutamate during the interpulse intervals. Once a pyramidal neurone had been impaled the glutamate electrode was gently lowered through the dendritic layer of the CA1 region until a maximal response to the ionophoretic pulses was observed in the neurone. The bathing medium was then changed to one containing 0·5 mM-$CaCl_2$ and 3·5 mM-$MgCl_2$. After synaptic blockade was achieved, as evidenced by the disappearance of the e.p.s.p. upon s.r. stimulation (usually 4–8 min

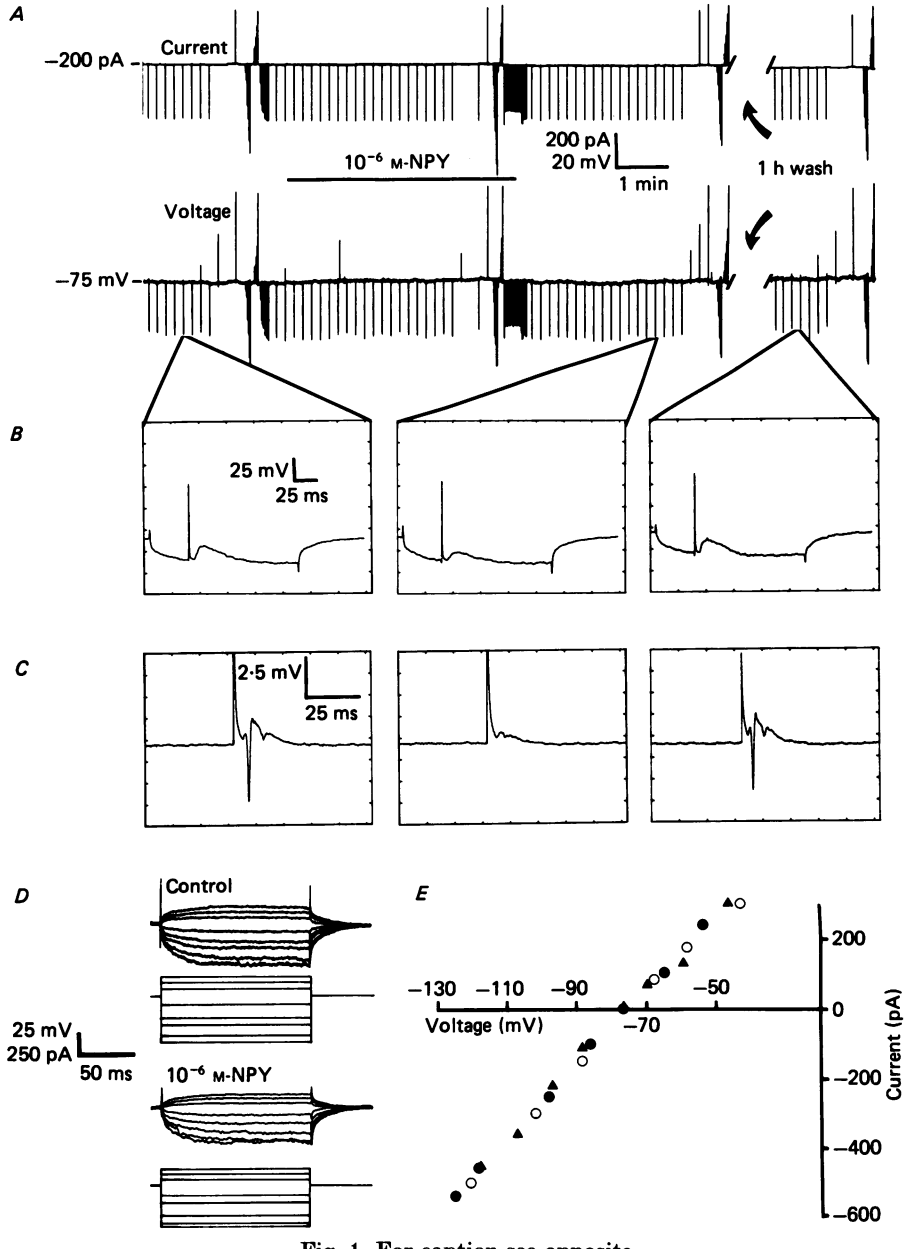

Fig. 1. For caption see opposite.

after the change in solution), NPY was bath-applied as before in the low-calcium, high-magnesium ACSF, and the potential change in response to the pulses of glutamate monitored.

All numerical data are presented as means ± S.E. of the means. Neurones were used as their own controls for statistical comparisons of NPY effects. Statistical comparisons were performed using a Student's paired t statistic, unless otherwise noted. Neurone input resistance was calculated from the slope of a least-squares regression line fitted to the data. Slope resistances were compared pairwise using an F test for comparing slopes of regression lines (Sokal & Rohlf, 1981). All data presented are from preparations which showed a recovery from the effects of NPY application.

RESULTS

The results were based on data from intracellular recordings of thirty-seven neurones under different experimental circumstances. Neurones sometimes received spontaneous excitatory input, requiring the injection of hyperpolarizing current to maintain membrane potential below action potential threshold during the experiment, to avoid contamination by residual currents induced by action potentials. The neurones selected for study met the following minimum criteria: resting membrane potential of more than 50 mV, slope input resistance of more than 35 MΩ, action potential amplitude of more than 70 mV, action potential overshoot of more than +20 mV, and action potential duration, measured at the half-amplitude of the first action potential evoked by a depolarizing current pulse, of less than 1·10 ms. These data compared favourably with properties of CA1 pyramidal neurones *in vitro* reported elsewhere (cf. Turner & Schwartzkroin, 1984).

Effects of NPY: post-synaptic properties

The bath application of 10^{-6} M-NPY did not significantly alter any of the properties of the post-synaptic neurone measured, based upon paired statistical comparisons of each neurone's properties before and during the effects of NPY. The resting membrane potential ($P > 0{\cdot}20$, $n = 11$; Fig. 1*A*), slope input resistance ($P > 0{\cdot}50$, $n = 11$; Fig. 1*D*), action potential amplitude ($P > 0{\cdot}90$, $n = 9$), action potential overshoot ($P > 0{\cdot}90$, $n = 9$) and action potential duration ($P > 0{\cdot}50$, $n = 9$) were all unaffected by the application of 10^{-6} M-NPY to the preparation. In addition, the number of action potentials evoked by identical current pulses was unaffected by NPY at this concentration ($P > 0{\cdot}50$, $n = 9$; Fig. 2). As can be seen in Fig. 2*B*, the initial membrane voltage trajectories in response to depolarizing current pulses also were unaffected by 10^{-6} M-NPY. Measurements of the threshold voltage for action potential initiation, elicited either by injection of current or by

Fig. 1. Summary of a typical experiment showing the effect of neuropeptide Y (NPY) on extracellularly and intracellularly recorded potentials in area CA1 of rat hippocampal slice. *A*, upper trace: intracellular current. Downward deflexions indicate passage of hyperpolarizing current through electrode. Lower trace: intracellular voltage. NPY was bath-applied at 10^{-6} M during the time indicated by the horizontal bar. Wash-out with control solution began immediately after NPY application ended. *B*, oscilloscope traces of intracellular voltage records from CA1 pyramidal neurone during hyperpolarizing pulse and e.p.s.p. elicited by stratum radiatum (s.r.) stimulation (upward deflexion is the stimulus shock artifact) 35 ms after the start of the hyperpolarizing pulse. Note reduction in e.p.s.p. amplitude at time of greatest effect of NPY, about 3 min after wash was initiated (middle trace), and recovery, following 1 h wash-out (right-hand trace). *C*, oscilloscope traces of extracellular voltage records from CA1 cell body layer near the impaled neurone. Responses are to the same s.r. stimuli which elicited the e.p.s.p.s shown in *B*. Upward deflexion is the stimulus shock artifact. Note reduction in population spike amplitude caused by NPY (middle trace), and its recovery after 1 h wash-out (right-hand trace). *D*, changes in membrane potential of neurone in response to hyperpolarizing and depolarizing current pulses. Upper two traces: control. Lower two traces: at time of maximum effect of NPY. Upper trace of each pair is voltage, lower trace, current record. *E*, plot of current–voltage relationship from records illustrated in *D*. ●, control, ▲, 10^{-6} M-NPY, ○, 1 h wash-out (not shown in *D*).

stimulation of the stratum radiatum, showed no change upon application of 10^{-6} M-NPY ($P > 0{\cdot}50$, $n = 3$).

Synaptic properties: e.p.s.p.

Stimulation of the s.r. induced a short-latency e.p.s.p. in CA1 neurones (Fig. 1*B*). The bath application of 10^{-6} M-NPY caused a smoothly progressive (2–3 min to

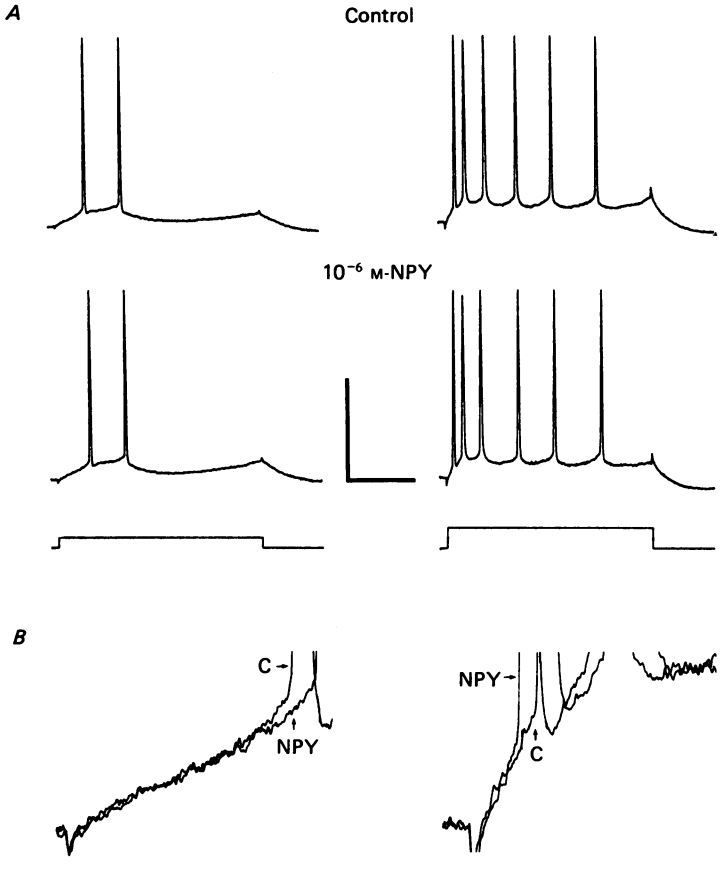

Fig. 2. Action potentials of a CA1 pyramidal neurone in response to the injection of pulses of depolarizing current. Membrane potential of neurone was −65 mV. *A*, action potentials elicited in control (upper traces) and 10^{-6} M-NPY (middle traces) by pulses of 100 (left-hand column) and 200 pA (right-hand column), indicated by the current (lower) traces. *B*, digital magnification of the voltage traces in *A*, which are superimposed for comparison of the initial voltage trajectories in response to the pulses of depolarizing current. 10^{-6} M-NPY had no consistent effect on the latency of the first action potential, or on any other action potential parameter measured. C, control, NPY, 10^{-6} M-NPY. Calibration: *A*, 50 mV, 1000 pA, 50 ms; *B*, 3·125 mV, 6·25 ms.

onset, 5–8 min to maximum) decrease in the amplitude of the e.p.s.p., to $59{\cdot}97 \pm 4{\cdot}78$ % ($n = 12$) of control amplitudes (Figs. 1*B*, 3*A* and 4). Wash-out of NPY at this concentration, which required 1–1·5 h (Colmers *et al.* 1985), allowed the e.p.s.p. to return to near control amplitudes (Figs. 1*B* and 3*A*). Examination of the stimulus–response relationship of the e.p.s.p. amplitude (Fig. 3*A*) demonstrated that

maximum e.p.s.p. amplitude was more strongly affected by NPY than were e.p.s.p. elicited by stimuli closer to threshold. Because of the variability in threshold and the range of stimulus voltages required for each different preparation, this relationship was not quantified, but the qualitative effects were very similar in every case. Experiments conducted at a higher temperature (37 °C, $n = 2$) showed no qualitative difference in this effect of NPY (Fig. 3*B*).

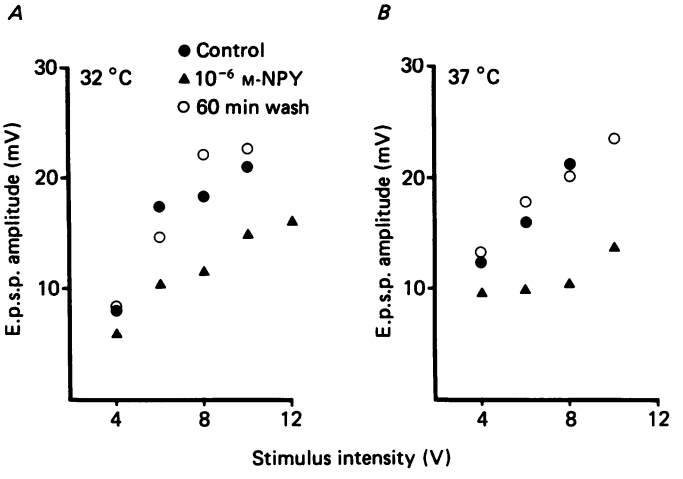

Fig. 3. Effect of 10^{-6} M-NPY on the stimulus–response relationship of intracellularly recorded e.p.s.p. elicited by stimulation of the s.r. at the voltages shown. *A*, data from an experiment performed at 32 °C. *B*, of data from a different experiment, performed at 37 °C. ●, control; ▲, at time of maximum NPY effect; ○, 60 min wash-out.

Preliminary experiments established that the effects of NPY are reproducible upon wash-out and reapplication. Despite the long wash-out times required it was frequently possible to apply two or more concentrations of NPY while recording from a given neurone. Partial dose–response relationships established on such neurones gave results similar to those seen between neurones.

A dose–response relationship was constructed for the effect of NPY on the e.p.s.p. evoked in CA1 pyramidal neurones by s.r. stimulation (Fig. 4). The values plotted in Fig. 4 were taken from stimulus voltages approximately in the middle of the stimulus–response relationship for each neurone. Concentrations of 10^{-8}, 10^{-7}, 3×10^{-7} and 10^{-6} M-NPY were tested. While 10^{-8} and 10^{-7} M-NPY were not significantly effective in altering the amplitude of the e.p.s.p. from control values ($P > 0{\cdot}25$ and $P > 0{\cdot}20$, respectively), 3×10^{-7} M and 10^{-6} M were effective in reducing the e.p.s.p. amplitude ($P < 0{\cdot}05$ and $P < 0{\cdot}05$, respectively). The dose–response relationship was relatively steep between 10^{-7} and 10^{-6} M. Experiments were not conducted at higher peptide concentrations.

Synaptic properties: population spike

The application of 10^{-6} M-NPY caused a reduction of the population spike in CA1 to $26{\cdot}49 \pm 6{\cdot}36\%$ of control (Figs. 1*C*, 5*A* and 6). The population spike amplitude returned to near control levels after wash-out of the peptide (Figs. 1*C* and 5*A*). As

with the e.p.s.p., the maximum population spike amplitude in response to a strong stimulus was more greatly affected than was the population spike elicited by stimuli near threshold. The population spike was a more sensitive measure of NPY's action than was the intracellularly recorded e.p.s.p. (compare Figs. 3*A* and 5*A*). In the

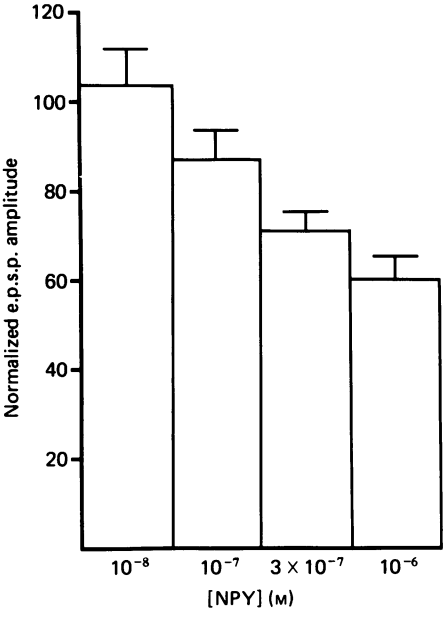

Fig. 4. Concentration dependence of the effect of NPY on the amplitude of the e.p.s.p. evoked in CA1 pyramidal neurones by s.r. stimulation. Data were normalized by setting the amplitude of the control e.p.s.p. equal to 100. For 10^{-8} M-NPY, $P > 0{\cdot}25$, $n = 6$; for 10^{-7} M-NPY, $P > 0{\cdot}20$, $n = 6$; for 3×10^{-7} M-NPY, $P < 0{\cdot}05$, $n = 9$; for 10^{-6} M-NPY, $P < 0{\cdot}05$, $n = 12$.

experiments conducted at 37 °C the effects of NPY application were qualitatively similar to those found at 32 °C (compare Figs. 5*A* and 5*B*).

The dose–response relationship for the effect of NPY on the population spike amplitude is shown in Fig. 6. The values plotted were taken from the same stimuli as elicited the data plotted in Fig. 4. The results were similar to those found for the e.p.s.p.: 10^{-8} M was not significantly effective ($P > 0{\cdot}25$), while 10^{-7} M-NPY was only marginally effective ($P < 0{\cdot}1$). However, 3×10^{-7} and 10^{-6} M were significantly effective in reducing the population spike amplitude ($P < 0{\cdot}025$ and $P < 0{\cdot}005$, respectively).

Because it appeared that NPY was more potent in depressing population spike amplitude than e.p.s.p. amplitude, we examined the relationship between these two variables in two different ways. For nine cells a graphic plot was made of the relationship between the amplitudes of the intracellularly recorded e.p.s.p.s and simultaneously recorded population spike. Application of 10^{-6} M-NPY to the preparation did not visibly affect this relationship. Because both the e.p.s.p. and the population spike reflect somatic events, we performed a second series of three

experiments in which we could more directly compare dendritic and somatic potentials. The extracellular field e.p.s.p. was therefore recorded with a second extracellular electrode inserted into the corresponding apical dendritic region of CA1 (Fig. 7). This field e.p.s.p. reflects the e.p.s.p.s generated in the apical dendrites of

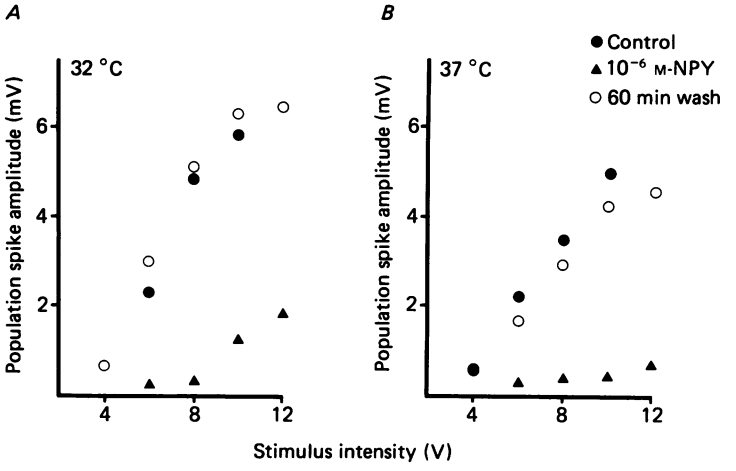

Fig. 5. Effect of 10^{-6} M-NPY on the stimulus–response relationship of the extracellular population spike, recorded in area CA1 near the neurones illustrated in Fig. 3, elicited by stimulation of the s.r. at the voltages indicated. Data shown were taken simultaneously with data shown in Fig. 3. *A*, data from an experiment performed at 32 °C. *B*, data from an experiment performed at 37 °C. ●, control; ▲, at time of greatest effect of NPY; ○, 60 min wash-out.

a large number of pyramidal neurones (Andersen, Silfvenius, Sundberg, Sveen & Wigstrom, 1978). The results of these experiments were consistent with those obtained from the intracellular experiments, in that NPY did not affect the relationship between population spike and e.p.s.p. amplitudes (Fig. 7).

Glutamate ionophoresis

The above data suggested that NPY acted presynaptically to modulate the release of excitatory neurotransmitter in the s.r. to CA1 pathway. However, the experiments did not exclude the possibilities that NPY had either a direct effect on the post-synaptic receptor for the excitatory neurotransmitter, or that it could locally decrease the membrane resistance of the dendrites or dendritic spines onto which the afferent terminals synapse, which could thus decrease the effect of a given synaptic input to the dendrites or dendritic spines. To examine these alternatives, pulses of glutamate, a putative excitatory transmitter in the Schaffer collateral inputs to CA1 (Wierasko, 1983), were ionophoretically applied to the apical dendrites of impaled CA1 neurones where the fibres of the s.r. terminate. To eliminate any contribution from other neurones which NPY or glutamate might activate, the experiments were conducted under conditions where synaptic transmission was eliminated, using low-calcium and high-magnesium concentrations to effect this blockade.

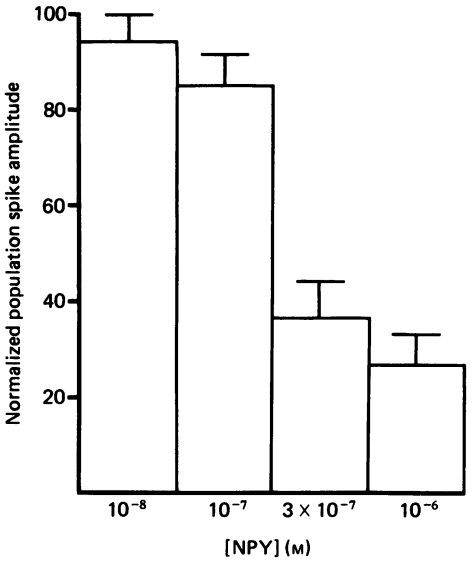

Fig. 6. Concentration dependence of the effect of NPY on the amplitude of the extracellularly recorded population spike evoked in area CA1 by s.r. stimulation. Data are from the same experiments as those plotted in Fig. 5. Data were normalized by setting the amplitude of the control population spike equal to 100. For 10^{-8} M-NPY, $P > 0{\cdot}25$, $n = 6$; for 10^{-7} M-NPY, $P > 0{\cdot}20$, $n = 6$; for 3×10^{-7} M-NPY, $P < 0{\cdot}025$, $n = 9$; for 10^{-6} M-NPY, $P < 0{\cdot}005$, $n = 12$.

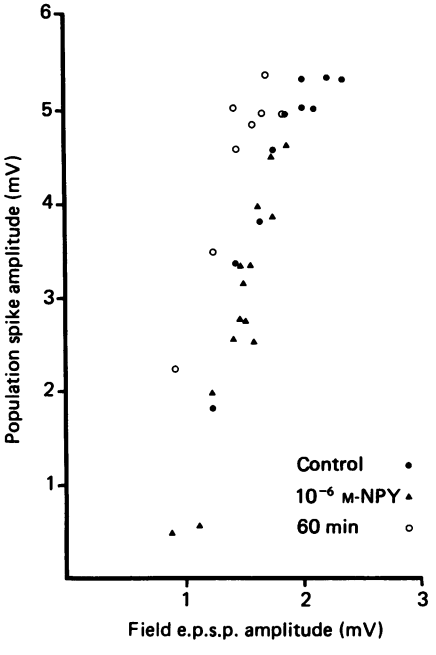

Fig. 7. Relationship between the amplitudes of the population spike and field e.p.s.p., recorded extracellularly from the apical dendritic region of area CA1. Application of 10^{-6} M-NPY (▲), while reducing the amplitudes both of the e.p.s.p. and population spike, did not alter the relationship from that seen in control (●), 60 min wash-out (○).

Results from one such experiment are illustrated in Fig. 8. Fig. 8*A*1 shows the e.p.s.p. evoked in the CA1 neurone in ACSF, while Fig. 8*B*1 shows the averaged response of the neurone to eight ionophoretic pulses of glutamate on different time and voltage scales. Subsequent replacement of the ACSF with a medium containing

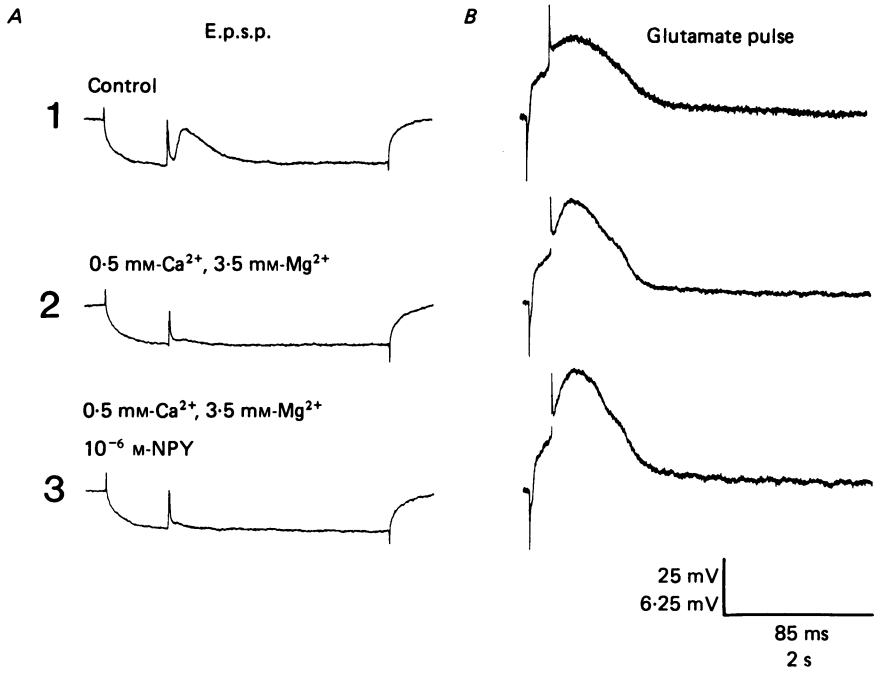

Fig. 8. Absence of effect by 10^{-6} M-NPY on the response of CA1 pyramidal neurone to a pulse of glutamate applied ionophoretically to its dendrites during blockade of synaptic transmission. Membrane potential of neurone was −71 mV. *A*, e.p.s.p. evoked, during a pulse of hyperpolarizing current passed via the bridge circuit, by stimulation of the s.r. 35 ms after start of hyperpolarizing pulse (upward deflexion is stimulus shock artifact). *A*1, in control medium. *A*2, in medium containing 0·5 mM-Ca^{2+} and 3·5 mM-Mg^{2+}. Note that the e.p.s.p. is abolished by this treatment. *A*3, in medium containing 0·5 mM-Ca^{2+}, 3·5 mM-Mg^{2+} and 10^{-6} M-NPY. *B*, voltage response of neurone to a 250 ms, 500 nA pulse of glutamate applied to its dendrites. Each trace is the average of eight responses. Onset and offset of ionophoretic current marked by vertical deflexions on rising phase of potential response. *B*1, in control medium. *B*2, in medium containing 0·5 mM-Ca^{2+} and 3·5 mM-Mg^{2+}. *B*3, in medium containing 0·5 mM-Ca^{2+}, 3·5 mM-Mg^{2+} and 10^{-6} M-NPY. Note different time and voltage scales for *A* (upper values), and *B* (lower values).

0·5 mM-$CaCl_2$ and 3·5 mM-$MgCl_2$ caused the elimination of the synaptic response (Fig. 8*A*2), while there was a slight increase in the response to the glutamate-pulse-induced potential change (Fig. 8*B*2). Application of 10^{-6} M-NPY in the low-calcium, high-magnesium ACSF did not affect a significant decrease in the amplitude of the glutamate pulse (Fig. 8*B*3).

The results from seven such experiments showed that the glutamate response was not significantly affected by NPY. The glutamate response in the presence of 10^{-6} M-NPY was $99{\cdot}29 \pm 11{\cdot}12$ % of control values ($P > 0{\cdot}90$). Similar experiments,

conducted in the presence of synaptic transmission, gave qualitatively similar results.

DISCUSSION

This is the first report to our knowledge on the action of the major brain peptide, NPY, on the membrane physiology of neurones of the central nervous system. We have shown that application of NPY to the *in vitro* rat hippocampal slice reduces, in a concentration-dependent manner, the amplitude of the e.p.s.p. evoked in CA1 by s.r. stimulation. This profound effect of NPY on synaptic transmission between fibres of s.r. and CA1 pyramidal neurones is unlikely to result from an action on the post-synaptic CA1 cells, as measures of the passive and active properties of these neurones, including input resistance, resting membrane potential, action potential threshold and other action potential parameters, were unaffected by the peptide. The relationship between e.p.s.p. and population spike amplitudes was unaffected by NPY, indicating that the ability of these neurones to translate a synaptically generated excitatory input into an action potential is not impaired by NPY. In addition, NPY had no effect on the post-synaptic neurones' responses to ionophoretic application of glutamate to the dendrites, indicating that NPY does not interfere with the dendritic glutamate receptors or reduce the input resistance of the dendrites or dendritic spines of the post-synaptic neurones. Thus, neither the transfer of electrotonic potential from the dendrites to the soma, the ability of the neurones to translate dendritic depolarizations into action potentials, nor any other detectable membrane electrical property of the post-synaptic CA1 pyramidal neurones, is affected by NPY. The evidence from this study therefore strongly supports our (Colmers *et al.* 1985) hypothesis that NPY acts presynaptically in this pathway of the hippocampus to decrease excitatory transmission.

This evidence is in accord with some reports of presynaptic inhibitory effects by NPY on synaptic transmission in peripheral tissues. In the vas deferens, it has been demonstrated that NPY inhibits both the fast twitch and sustained tonic response to electrical stimulation, without affecting the contractile response to exogenously applied putative transmitter substances, (Allen *et al.* 1982; Ohhashi & Jacobowitz, 1983; Lundberg *et al.* 1984; Lundberg & Stjarne, 1984).

The experiments in this study did not identify the site or sites of action of NPY in the hippocampus. Although a number of possibilities exist, two appear to be most likely. The first is that NPY acts directly on the presynaptic terminal of the afferent neurones providing excitatory input onto CA1 pyramidal neurones, causing a reduction in neurotransmitter release. In this context, it is noteworthy that a recent report has shown that NPY binds specifically and with a high affinity to brain synaptosomes (Saria, Theodorsson-Norheim & Lundberg, 1985). The second is that NPY works indirectly to excite inhibitory interneurones presynaptic to the terminals, axons or cell bodies of the neurones providing excitatory input to the CA1 pyramidal neurones. Because there was no change due to NPY in the input resistance of the post-synaptic CA1 neurones observed, it appears unlikely that inhibitory interneurones making direct synaptic contact with the somata of the CA1 pyramidal neurones are activated by NPY. It cannot, however, be excluded from these experiments that

NPY may also affect the feed-forward or recurrent feed-back inhibitory post-synaptic potentials, because these would have been contaminated by the e.p.s.p. in these experiments (Anderson, Eccles & Loyning, 1964; MacVicar & Dudek, 1980). Further experiments will address these possibilities.

Our data indicate that NPY reduces the orthodromically evoked population spike in CA1 to a greater extent than the e.p.s.p. recorded from the pyramidal neurones in this area (compare Figs. 4 and 6). The population spike is a measure of the extracellular field potential generated by simultaneous action potentials in a great number of neurones (Andersen, Bliss & Skrede, 1971). The extracellularly recorded field e.p.s.p. is a reflexion of the post-synaptic dendritic response of a great number of neurones to the excitatory synaptic input caused by s.r. stimulation, and bears a linear relationship to the intracellularly recorded e.p.s.p. (Andersen *et al.* 1978). The relationship between the amplitudes of the p.s. and the field e.p.s.p. is sigmoidal, with a relatively steep slope in the middle portion of the relationship (Andersen *et al.* 1978, 1980). NPY, by reducing the amount of excitatory input to the neurones, shifts the relationship between e.p.s.p. and population spike into the steep part of the curve. Therefore a small reduction in e.p.s.p. amplitude causes a disproportionate reduction in the number of neurones achieving action potential threshold, and thus causes a relatively greater reduction in population spike amplitude. As this study has demonstrated that the relationship between e.p.s.p. and population spike amplitudes was unaffected by NPY, the reduction in e.p.s.p. amplitude is sufficient to explain the differences as seen in NPY's effects on the two variables.

The effects of NPY at the highest concentration tested (10^{-6} M) required 1–1·5 h for complete reversal, as was seen earlier (Colmers *et al.* 1985). This is a much longer period than that required for other peptides such as somatostatin (Pittman & Siggins, 1981) or oxytocin (Muhlethaler, Charpak & Dreifuss, 1984). It would also appear that neuronal responses to NPY do not desensitize, as has been reported for NPY in the periphery (Ohhashi & Jacobowitz, 1983). Exogenously applied NPY has been shown to have long-lasting effects on the physiology of peripheral tissues (Lundberg *et al.* 1982; Fuxe *et al.* 1983; Lundberg & Stjarne, 1984) and of the central nervous system (Carter, Vallejo & Lightman, 1985; Potter, 1985; Stanley & Liebowitz, 1985). This raises the possibility that endogenous NPY has rather long-term effects when released by neurones in the intact brain. Because NPY appears to be co-stored (and presumably co-released) by neurones which contain other neurotransmitters, such as NA and GABA, it is quite possible that NPY's long-term effects may interact with the shorter-term effects of these other neurotransmitters. It is of interest to note that the effects of NPY on CA1 pyramidal neurones differ markedly from those of the substances co-localized in neurones with it. NA, GABA, and the peptide somatostatin (Hendry, Jones & Emson, 1984; Chronwall, Chase & O'Donohue, 1984) have all been shown to have post-synaptic actions on CA1 neurones (Pittman & Siggins, 1981; Alger & Nicoll, 1982; Madison & Nicoll, 1986). It is conceivable, however, that co-release of NPY by neurones containing any of these substances could augment their post-synaptic inhibitory effects in inhibiting excitatory transmission presynaptically.

The authors would like to thank Ms Laurel Franklin for her expert technical assistance. The work was supported by the M.R.C. of Canada. W.F.C. is an Alberta Heritage Foundation for Medical Research Fellow. Q.J.P. is an M.R.C. Scientist and A.H.F.M.R. Scholar.

REFERENCES

ADRIAN, T. E., ALLEN, J. M., BLOOM, S. R., GHATEI, M. A., ROSSOR, M. N., ROBERTS, G. W., CROW, T. J., TATEMOTO, K. & POLAK, J. M. (1983). Neuropeptide Y distribution in human brain. *Nature* **306**, 584–586.

ALBERS, H. E. & FERRIS, C. F. (1984). Neuropeptide Y: role in light–dark entrainment of hamster circadian rhythms. *Neuroscience Letters* **50**, 163–168.

ALGER, B. E. & NICOLL, R. A. (1982). Pharmacological evidence for two kinds of GABA receptor on rat hippocampal pyramidal cells studied *in vitro*. *Journal of Physiology* **325**, 125–141.

ALLEN, J. M., ADRIAN, T. E., TATEMOTO, K., POLAK, J. M., HUGHES, J. & BLOOM, S. R. (1982). Two novel related peptides, neuropeptide Y (NPY) and peptide YY (PYY) inhibit the contraction of the electrically stimulated mouse vas deferens. *Neuropeptides* **3**, 71–77.

ALLEN, Y. S., ADRIAN, T. E., ALLEN, J. M., TATEMOTO, K., CROW, T. J., BLOOM, S. R. & POLAK, J. M. (1983). Neuropeptide Y distribution in the rat brain. *Science* **221**, 877–879.

ANDERSEN, P., BLISS, T. V. P. & SKREDE, K. K. (1971). Unit analysis of hippocampal population spikes. *Experimental Brain Research* **13**, 208–221.

ANDERSEN, P., ECCLES, J. C. & LOYNING, Y. (1964). Pathway of postsynaptic inhibition in the hippocampus. *Journal of Neurophysiology* **27**, 608–619.

ANDERSEN, P., SILFVENIUS, H., SUNDBERG, S. H., SVEEN, O. & WIGSTROM, H. (1978). Functional characteristics of unmyelinated fibres in the hippocampal cortex. *Brain Research* **144**, 11–18.

ANDERSEN, P., SUNDBERG, S. H., SVEEN, O., SWANN, J. W. & WIGSTROM, H. (1980). Possible mechanisms for long-lasting potentiation of synaptic transmission in hippocampal slices from guinea-pigs. *Journal of Physiology* **302**, 463–482.

CARTER, D. A., VALLEJO, M. & LIGHTMAN, S. L. (1985). Cardiovascular effects of neuropeptide Y in the nucleus tractus solitarius of rats: relationship with noradrenaline and vasopressin. *Peptides* **6**, 421–425.

CHRONWALL, B. M., CHASE, T. N. & O'DONOHUE, T. L. (1984). Coexistence of neuropeptide Y and somatostatin in rat and human cortical and rat hypothalamic neurons. *Neuroscience Letters* **52**, 213–217.

CHRONWALL, B. M., DIMAGGIO, D. A., MASSARI, V. J., PICKEL, V. M., RUGGIERO, D. A. & O'DONOHUE, T. L. (1985). The anatomy of neuropeptide Y containing neurons in the rat brain. *Neuroscience* **15**, 1159–1181.

COLMERS, W. F., LUKOWIAK, K. D. & PITTMAN, Q. J. (1985). Neuropeptide Y reduces orthodromically evoked population spike in rat hippocampal CA1 by a possibly presynaptic mechanism. *Brain Research* **346**, 404–408.

EDVINSSON, L., EKBLAD, E., HÅKANSON, R. & WAHLESTEDT, C. (1984). Neuropeptide Y potentiates the effect of various vasoconstrictor agents on rabbit blood vessels. *British Journal of Pharmacology* **83**, 519–525.

EDVINSSON, L., EMSON, P., MCCULLOCH, J., TATEMOTO, K. & UDDMAN, R. (1983). Neuropeptide Y: cerebrovascular innervation and vasomotor effects in the cat. *Neuroscience Letters* **43**, 79–84.

EKBLAD, E., EDVINSSON, L., WAHLESTEDT, C., UDDMAN, R., HÅKANSON, R. & SUNDLER, F. (1984). Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibres. *Regulatory Peptides* **8**, 225–235.

EVERITT, B. J., HOKFELT, T., TERENIUS, L., TATEMOTO, K., MUTT, V. & GOLDSTEIN, M. (1984). Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. *Neuroscience* **11**, 443–462.

FUXE, K., AGNATI, L. F., HARFSTRAND, A., ZINI, I., TATEMOTO, K., PICH, E. M., HOKFELT, T., MUTT, V. & TERENIUS, L. (1983). Central administration of neuropeptide Y induces hypotension, bradypnea, and EEG synchronization in the rat. *Acta physiologica scandinavica* **118**, 189–192.

GU, J., POLAK, J. M., ALLEN, J. M., HUANG, W. M., SHEPPARD, M. N., TATEMOTO, K. & BLOOM, S. R. (1984). High concentrations of a novel peptide, neuropeptide Y, in the innervation of mouse and rat heart. *Journal of Histochemistry and Cytochemistry* **32**, 467–472.

HENDRY, S. H. C., JONES, E. G., DEFELIPE, J., SCHMECHEL, D., BRANDON, C. & EMSON, P. C. (1984). Neuropeptide-containing neurons of the cerebral cortex are also GABAergic. *Proceedings of the National Academy of Sciences of the U.S.A.* **81**, 6526–6530.

HENDRY, S. H. C., JONES, E. G. & EMSON, P. C. (1984). Morphology, distribution, and synaptic relations of somatostatin- and neuropeptide Y-immunoreactive neurons in rat and monkey neocortex. *Journal of Neuroscience* **4**, 2497–2517.

KALRA, S. P. & CROWLEY, W. R. (1984). Norepinephrine-like effects of neuropeptide Y on LH release in the rat. *Life Sciences* **35**, 1173–1176.

LUNDBERG, J. M., HUA, X. Y. & FRANCO-CERECEDA, A. (1984). Effects of neuropeptide Y (NPY) on mechanical activity and neurotransmission in the heart, vas deferens and urinary bladder of the guinea pig. *Acta physiologica scandinavica* **121**, 325–332.

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

LUNDBERG, J. M. & TATEMOTO, K. (1982). Pancreatic polypeptide family (APP, BPP, NPY, and PYY) in relation to sympathetic vasoconstriction resistant to α-adrenoreceptor blockade. *Acta physiologica scandinavica* **116**, 393–402.

LUNDBERG, J. M., TERENIUS, L., HOKFELT, T. & GOLDSTEIN, M. (1983). High levels of neuropeptide Y in peripheral noradrenergic neurons in various mammals including man. *Neuroscience Letters* **42**, 167–172.

LUNDBERG, J. M., TERENIUS, L., HOKFELT, T., MARTLING, C. R., TATEMOTO, K., MUTT, V., POLAK, J., BLOOM, S. R. & GOLDSTEIN, M. (1982). Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. *Acta physiologica scandinavica* **116**, 477–480.

MACVICAR, B. A. & DUDEK, F. E. (1980). Local synaptic circuits in rat hippocampus: interactions between pyramidal cells. *Brain Research* **184**, 220–223.

MADISON, D. V. & NICOLL, R. A. (1986). Actions of noradrenaline recorded intracellularly in rat hippocampal CA1 neurones, *in vitro*. *Journal of Physiology* **372**, 221–244.

MUHLETHALER, M., CHARPAK, S. & DREIFUSS, J. J. (1984). Contrasting effects of neurohypophyseal peptides on pyramidal and nonpyramidal neurones in the rat hippocampus. *Brain Research* **308**, 97–107.

OHHASHI, T. & JACOBOWITZ, D. M. (1983). The effects of pancreatic polypeptides and neuropeptide Y on the rat vas deferens. *Peptides* **4**, 381–386.

PITTMAN, Q. J. & SIGGINS, G. R. (1981). Somatostatin hyperpolarizes hippocampal pyramidal cells *in vitro*. *Brain Research* **221**, 402–408.

POTTER, E. K. (1985). Prolonged non-adrenergic inhibition of cardiac vagal action following sympathetic stimulation: neuromodulation by neuropeptide Y? *Neuroscience Letters* **54**, 117–121.

SARIA, A., THEODORSSON-NORHEIM, E. & LUNDBERG, J. M. (1985). Evidence for specific neuropeptide Y-binding sites in rat brain synaptosomes. *European Journal of Pharmacology* **107**, 105–107.

SOKAL, R. R. & ROHLF, F. J. (1981). *Biometry*, 2nd edn. San Francisco: W. H. Freeman.

STANLEY, B. G. & LIEBOWITZ, S. F. (1985). Neuropeptide Y injected in the paraventricular hypothalamus: a powerful stimulant of feeding behavior. *Proceedings of the National Academy of Sciences of the U.S.A.* **82**, 3940–3943.

SUNDLER, F., MOGHIMZADEH, E., HAKANSON, R., EKELUND, M. & EMSON, P. (1983). Nerve fibers in the gut and pancreas of the rat displaying neuropeptide-Y immunoreactivity. Intrinsic and extrinsic origin. *Cell and Tissue Research* **230**, 487–493.

TATEMOTO, K. (1982). Neuropeptide Y: complete amino acid sequence of the brain peptide. *Proceedings of the National Academy of Sciences of the U.S.A.* **79**, 5485–5489.

TURNER, D. A. & SCHWARTZKROIN, P. A. (1984). Passive electrotonic structure and dendritic properties of hippocampal neurones. In *Brain Slices*, ed. DINGLEDINE, R., pp. 25–50. New York: Plenum.

WIERASKO, A. (1983). Glutamic acid and aspartic acid as putative neurotransmitters: release and uptake studies on hippocampal slices. In *Neurobiology of the Hippocampus*, ed. SEIFERT, W., pp. 175–196. New York: Academic.

WILLOUGHBY, J. O. & BLESSING, W. W. (1985). Neuropeptide Y and endogenous noradrenaline stimulate secretion of vasopressin in the rat. *Neuroscience Abstracts* **11**, 1057.