J. Physiol. (1984), **351**, *pp.* 111–121
With 5 text-figures
Printed in Great Britain

SITES AND MECHANISMS OF ACTIONS OF ENKEPHALIN IN THE FELINE PARASYMPATHETIC GANGLION

BY Y. KATAYAMA AND S. NISHI*

*From the Department of Autonomic Physiology, Medical Research Institute, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101, and the * Department of Physiology, Kurume University School of Medicine, Kurume 830, Japan*

(*Received 19 July 1983*)

SUMMARY

1. Intracellular recordings were made *in vitro* from neurones of the cat parasympathetic ciliary ganglion with a current- or voltage-clamp technique.

2. (Met^5)enkephalin and (leu^5)enkephalin (10 nM to 10 μM) were applied by superfusion. Both caused a membrane hyperpolarization which persisted in a calcium-free/high-magnesium solution, and both reduced the amplitude of excitatory post-synaptic potentials (e.p.s.p.s). These actions of enkephalin were antagonized by naloxone.

3. The enkephalin-induced hyperpolarization was associated with an increase in membrane conductance, reversed in polarity at -90 mV and was not altered by changing external sodium and chloride concentrations. This indicates that the enkephalin hyperpolarization is due mainly to activation of potassium conductance.

4. Enkephalin decreased the mean quantal content of e.p.s.p.s recorded in low-calcium/high-magnesium solution, without changing quantal size. Furthermore, the increase in the frequency of miniature e.p.s.p.s after tetanic preganglionic stimulations was inhibited by enkephalin. Acetylcholine potentials were not altered by enkephalin. These findings suggest that enkephalin reduces transmitter release.

5. The experiments suggest that enkephalin may inhibit ganglionic transmission by both pre- and post-synaptic actions in a mammalian parasympathetic ganglion.

INTRODUCTION

(Leu^5)enkephalin (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975) has been localized by an immunohistochemical study in preganglionic nerve terminals in the avian ciliary ganglion (Erichsen, Reiner & Karten, 1982). This might imply involvement of enkephalin in synaptic transmission in this ganglion. Electrophysiological investigations with intracellular recordings have disclosed that enkephalin has two effects on mammalian neurones. It hyperpolarizes neurones of the myenteric plexus (North, Katayama & Williams, 1979; Morita & North, 1982) and the locus coeruleus nucleus (Pepper & Henderson, 1980; Williams, Egan & North, 1982), and it reduces transmitter release in the inferior mesenteric ganglion (Konishi, Tsunoo

& Otsuka, 1979). Morphine is also known to hyperpolarize the myenteric neurones (North & Tonini, 1977) and to inhibit transmitter release in the inferior mesenteric ganglion (Bornstein & Fields, 1979).

The parasympathetic ciliary ganglion sends post-ganglionic fibres to the iris sphincter and participates in regulating pupillary size. As is widely known, morphine induces myosis in the man and dog, whereas it produces mydriasis in the cat and monkey. Although the myosis induced by morphine in the dog is thought to be mediated via the central nervous system (see Lee & Wang, 1975), the possible involvement of the ciliary ganglion in the morphine-induced change in pupillary size is not known.

The present experiments were undertaken to examine whether or not enkephalin affected transmission in the ciliary ganglion of the cat. It was found that (met^5)- and (leu^5)enkephalin produced both a post-synaptic hyperpolarization and a presynaptic inhibition in this parasympathetic ganglion. These actions are discussed in relation to the morphine-induced mydriasis of the cat. Preliminary accounts of this work have been reported elsewhere (Katayama & Nishi, 1981).

METHODS

Adult cats of either sex were anaesthetized with α-chloralose (60 mg/kg, I.P.) and pentobarbitone (10 mg/kg, I.P.). The ciliary ganglia with the oculomotor and ciliary nerves were isolated and kept in a Krebs solution of the following composition (mM): NaCl, 117; KCl, 4·7; $CaCl_2$, 2·5; $MgCl_2$, 1·2; NaH_2PO_4, 1·2; $NaHCO_3$, 25; glucose, 11·5; gassed with 95% O_2 and 5% CO_2. Each ganglion was mounted in a recording chamber and was superfused with pre-warmed Krebs solution (36–37 °C). The volume of the chamber was approximately 1 ml and the flow rate of the solution was 2–4 ml/min.

Ganglion cells were impaled with glass micro-electrodes filled with 3 M-KCl or 2 M-K citrate. D.c. tip resistances of the electrodes were between 20 and 60 MΩ. Membrane potential was recorded by using a high-input-impedance pre-amplifier (WPI M701 or M707) and was displayed on an oscilloscope and on a pen-writing recorder chart. Transmembrane currents were passed through the recording electrode by means of a bridge circuit of the pre-amplifier. Neuronal input resistances were estimated by the amplitude of electrotonic potentials which were induced by transmembrane inward current pulses of 50–100 ms duration, repeated at 0·3 Hz. The resting membrane potential was determined by a sudden withdrawal of the micro-electrode from the cell.

Some experiments were made with a voltage-clamp method using double-barrelled electrodes. One of the barrels was used for recording, and the other was used for passing currents across the cell membrane. The double-barrelled electrodes were filled with 3 M-KCl, and only those with coupling resistances lower than 100 kΩ were used; this value was less than 2% of neuronal input resistance of ganglion cells (see Ginsborg & Kado, 1975). The voltage-clamp set-up consisted of the pre-amplifier mentioned above and of a high-gain differential amplifier described elsewhere (Katayama & Nishi, 1982).

Excitatory post-synaptic potentials (e.p.s.p.s) were evoked by electrical stimulation applied to preganglionic fibres of the oculomotor nerve using a suction electrode. Drugs were applied by superfusion. There was a time delay of approximately 30 s before the drug-containing solution reached the ganglion. The drugs used were (met^5)- and (leu^5)enkephalin (Peptide Institute, Osaka), acetylcholine (ACh) chloride (Wako), (+)-tubocurarine chloride (Wako), atropine sulphate (Wako) and naloxone chloride (a gift of du Pont, Endo Laboratories). All quantitative results are expressed as mean ± S.E. of the mean for the number of observations stated.

RESULTS

General observations

Stable intracellular recordings were obtained from ninety-six neurones (mainly from B cells; see Nishi & Christ, 1971). Neurones which had unstable fluctuations of membrane potential, a low resting potential (less negative than −50 mV for current-clamp study and −40 mV for voltage-clamp study) and/or a small action potential (less than 60 mV) were excluded. The input membrane resistance, measured at the resting membrane potential, was between 10 and 120 MΩ (39·2 ± 4·1 MΩ, $n = 38$). These values are considerably lower than those of rabbit ciliary ganglion cells (Johnson & Purves, 1981) but are comparable to the values of other mammalian parasympathetic ganglion cells (see Suzuki & Volle, 1979; Griffith, Gallagher & Shinnick-Gallagher, 1980). Preganglionic stimulation evoked e.p.s.p.s in almost all ganglion cells. These e.p.s.p.s were reversibly depressed by (+)-tubocurarine (3–10 μM) but not by atropine (3 μM) (see Perry & Talesnik, 1953). Other types of synaptically mediated potentials such as slow excitatory and inhibitory post-synaptic potentials were not observed even after stimuli comprising trains of pulses.

(Met^5)- and (leu^5)enkephalin (30 nM–10 μM) caused a membrane hyperpolarization or a reduction of e.p.s.p. amplitude or both. Both effects were antagonized by naloxone (1–10 μM).

Enkephalin hyperpolarization

Superfusion of either (leu^5)- or (met^5)enkephalin (30 nM to 10 μM) hyperpolarized the cell membrane in sixty of ninety-six cells. The hyperpolarization started 5 s after the enkephalin-containing solution reached the preparation and was usually maintained throughout the presence of enkephalin up to 5 min. During a prolonged application the response sometimes was not maintained in the presence of the peptide. The hyperpolarization was unchanged in calcium-free/6·0 mM-magnesium solutions ($n = 3$) and 0·5 mM-calcium/5·5 mM-magnesium solutions ($n = 2$); this suggests that enkephalin acts directly on the ganglion cell membrane. The amplitude of the hyperpolarization by a given concentration was different from cell to cell (maximum was 10 mV), but in a given neurone the enkephalin hyperpolarization was concentration dependent (Fig. 1). Repeated applications of enkephalin at concentrations higher than 3 μM often caused a progressive decline in the amplitude of the hyperpolarization. (Met^5)enkephalin was 5–10 times more potent than (leu^5)enkephalin (Fig. 1).

Enkephalin decreases input resistance. The enkephalin hyperpolarization was associated with a decrease in membrane resistance, which was apparent both as a reduction in the amplitude of the anelectrotonic potential (Fig. 1) and a decrease in the slope of the voltage–current relationship. The mean reduction of the slope resistance in ten neurones was 29 ± 7 %.

Enkephalin reversal potential. The ciliary ganglion cells often showed membrane rectification outside the range between −50 and −80 mV (Y. Katayama & S. Nishi, unpublished observation; see Fig. 2*A*). Such a characteristic made it difficult to obtain an accurate reversal potential for the enkephalin hyperpolarization by a current-clamp technique. Therefore, a voltage-clamp method was used to determine the reversal potential; the results are illustrated in Fig. 2. Enkephalin caused an

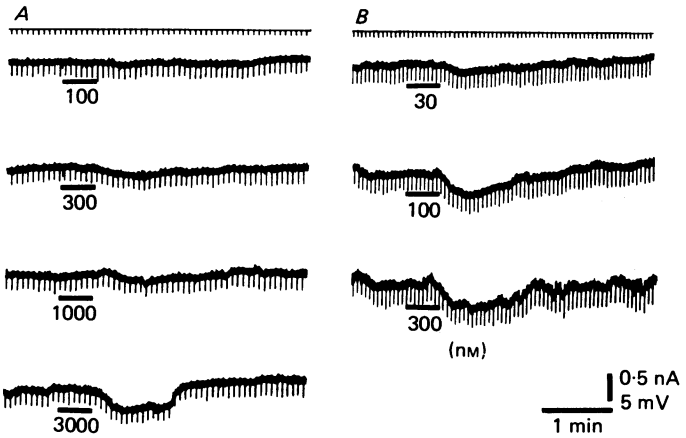

Fig. 1. Concentration-dependent hyperpolarization produced by enkephalin. During the periods indicated by bars, the superfusing solution which contained (leu^5)enkephalin (*A*) or (met^5)enkephalin (*B*) in the concentration indicated (nM) was applied. Both caused membrane hyperpolarizations which were dependent on the concentration applied. Top traces in *A* and *B* are records of hyperpolarizing current pulses (see Methods). There was a small decrease in the amplitude of anelectrotonic potentials during the enkephalin-induced hyperpolarization.

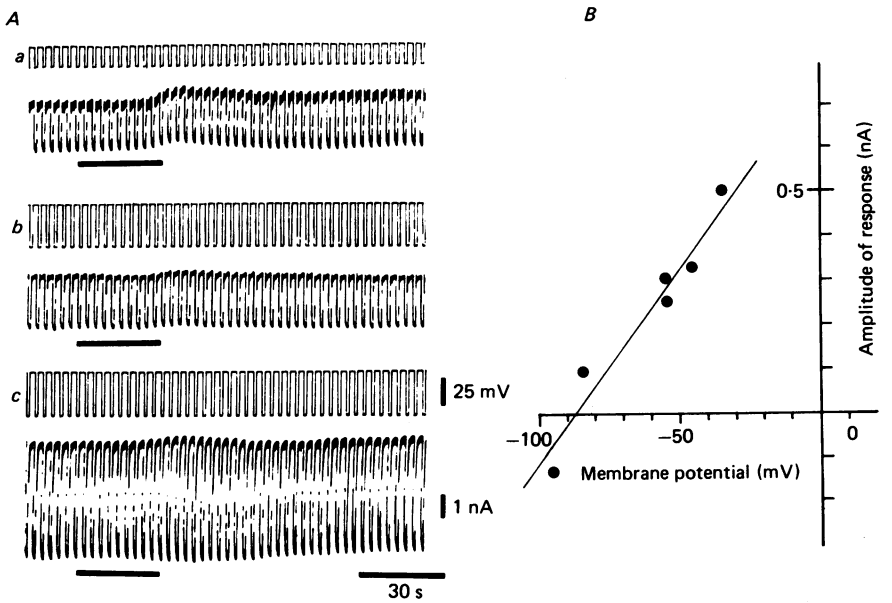

Fig. 2. The relationship between amplitude of enkephalin-induced response and membrane potential obtained from a voltage-clamped neurone. *A*, (met^5)enkephalin (3 μM) was applied with superfusion during the periods indicated by bars. Enkephalin-induced current responses were recorded at three holding levels; −35 mV (*a*), −45 mV (*b*) and −55 mV (*c*), respectively. The commanding pulse (duration: 1 s) of 20 mV (*a*) and 40 mV (*b* and *c*) was repeated at 0·3 Hz (upper traces). This neurone showed a property of marked membrane rectification, i.e. membrane conductance increased by membrane depolarization (*a*) and by hyperpolarization (*c*). *B*, peak amplitude of each current response (inward current: upward direction of ordinate) was plotted against membrane potential (abscissa). Values were taken from *A*. The reversal potential of this neurone was approximately −88 mV.

outward current when the cell was voltage clamped at its resting potential (−45 mV, upper envelope of the lower trace of *b* in Fig. 2*A*). The enkephalin-induced current became small when it was clamped at −85 mV (lower envelope of the lower trace of *b* in Fig. 2*A*). The outward current was associated with an increase in membrane conductance. The effect of enkephalin was also recorded at two other holding levels (−35 and −55 mV) while applying command pulses (*a* and *c* in Fig. 2*A*). When clamped at −35 mV, a large outward current was induced, whereas an inward current occurred at −95 mV (pulsing from −55 mV). The amplitude of the response was plotted against the membrane potential (Fig. 2*B*). The reversal potential of the response in this neurone was approximately −88 mV. Similar results were obtained from three other cells and the mean reversal potential was $-90{\cdot}2 \pm 2{\cdot}6$ mV ($n = 4$).

As noticed from Fig. 2*A*, slow relaxations of current in response to voltage pulses were sometimes observed. Although the nature of the slow relaxations was not clear, enkephalin apparently had no consistent effect on them (see Discussion).

Effects of changing extracellular ions. When the potassium concentration in the superfusing solution was lowered to 1·0 mM, the enkephalin hyperpolarization became substantially larger (about 1·5 times, $n = 3$). This occurred in spite of a hyperpolarization of the cell membrane (5–10 mV). The enkephalin hyperpolarization was still observed in a solution in which the sodium chloride of the Krebs solution was replaced with sucrose ($n = 2$).

Inhibition of synaptic transmission by enkephalin

(Met^5)- and (leu^5)enkephalin (30 nM to 10 μM) reduced the e.p.s.p.s in thirty-nine of fifty experiments. The degree of reduction of the e.p.s.p.s at a given enkephalin concentration varied from cell to cell. Although the inhibitory effects were most often observed for enkephalin concentrations higher than 1 μM, low concentrations of enkephalin occasionally induced a biphasic action (see below). The reduction of the e.p.s.p. induced by high concentrations (3–10 μM) became less marked during successive applications. A clear concentration dependence such as was observed for the enkephalin hyperpolarization (see Fig. 1) could not be detected consistently for the inhibitory action of enkephalin on the e.p.s.p.s.

Two mechanisms were considered for the inhibition of the e.p.s.p.s: one was a reduction of the amount of the transmitter liberated from the preganglionic fibres and the other was a decrease of the post-synaptic sensitivity to ACh.

Reduced amplitude of e.p.s.p.s. ACh depolarized the soma membrane when applied ionophoretically. The depolarization persisted in a calcium-free/high-magnesium solution. Fig. 3 shows the effect of (met^5)enkephalin on the e.p.s.p.s and on the ACh potentials. Enkephalin reduced the amplitude of the e.p.s.p.s by 50% but did not significantly alter the amplitude of the ACh potentials. (Met^5)enkephalin had no effect on the resting membrane potential of this neurone. This suggests that the site at which enkephalin depressed the e.p.s.p. is presynaptic. Usually the e.p.s.p.s recovered in amplitude 10–15 min after the removal of enkephalin from the superfusing solution. However, as shown in *c* of Fig. 3*A*, the e.p.s.p.s were sometimes larger after washing than those of the control (*a* of Fig. 3*A*). Such a biphasic action will be described below.

Decreased quantal content of e.p.s.p.s. Fig. 4 shows the amplitude distribution of the

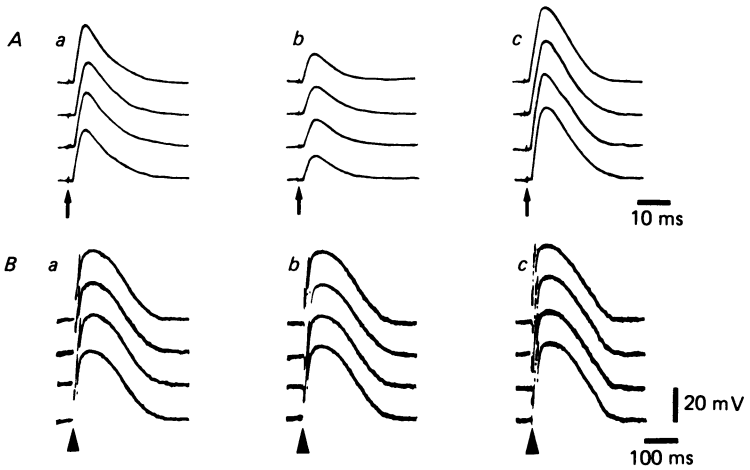

Fig. 3. Effects of enkephalin on e.p.s.p.s and ACh potentials. *A*, e.p.s.p.s were evoked by preganglionic supramaximal stimulations, indicated by arrows, before (*a*), 3 min after (*b*) beginning of (met^5)enkephalin application (3 μM) and 18 min after wash-out (*c*), respectively. *B*, ACh potentials were induced by ACh ionophoresis (100 nA for 20 ms, at triangles) before (*a*), during (*b*) and after (*c*) application of enkephalin. All of the records in *A* and *B* were taken in the presence of atropine (3 μM) from the same ganglion cell which was hyperpolarized to about −80 mV to prevent spike initiation. Enkephalin had no significant effect on membrane potential and resistance of this cell.

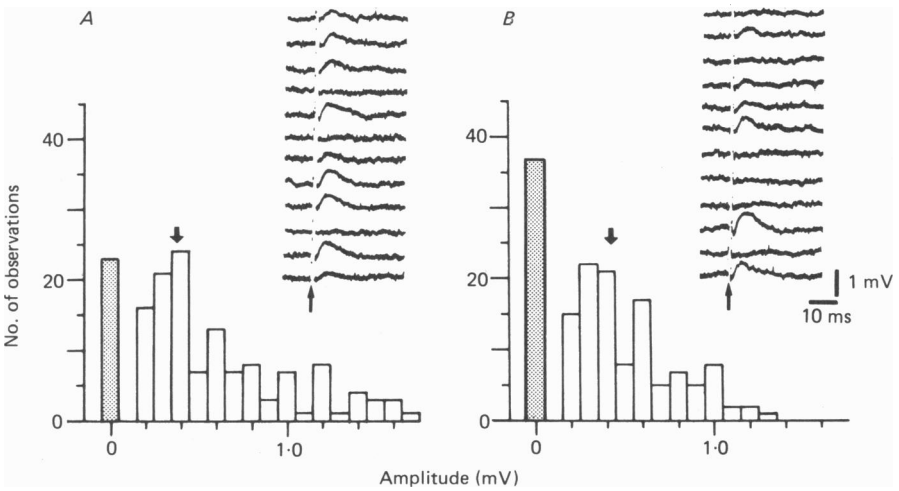

Fig. 4. Amplitude histograms of e.p.s.p.s recorded in low-calcium/high-magnesium solution. *A* and *B* were taken in control and in the presence of (met^5)enkephalin (3 μM), respectively. Insets of *A* and *B* show sample records of e.p.s.p.s which were evoked by preganglionic supramaximal stimulations at 1·0 Hz, indicated by arrows. The numbers of failures are indicated by stippled bars at zero amplitude in *A* and *B*. Arrows in histograms point values of quantal sizes.

e.p.s.p.s induced, in a single cell, by repeated preganglionic stimulations in a low-calcium/high-magnesium solution. In the control record, there were 23 failures in 149 stimulations (Fig. 4*A*). Assuming a Poisson distribution for transmitter release in this solution (in which the probability of transmitter release is lowered), we calculated an average quantal content of 1·9. During superfusion with enkephalin (3 μM), the number of failures increased to 37 in 151 stimulations (Fig. 4*B*) and the calculated mean quantal content fell to 1·4 from 1·9, i.e. by about 25 %. After washing

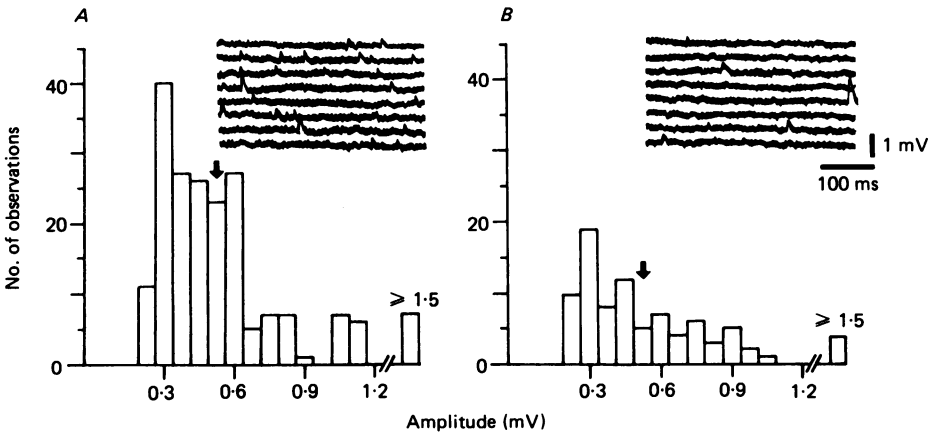

Fig. 5. Amplitude histograms of m.e.p.s.p.s recorded in control (*A*) and in the presence of (met^5)enkephalin (1 μM) (*B*). Each histogram describes the m.e.p.s.p.s recorded during a period of 60 s following tetanic preganglionic stimulations (20 Hz for 10 s) (see text). Insets of *A* and *B* illustrate sample records of m.e.p.s.p.s. There were 197 m.e.p.s.p.s in control (*A*) and 88 in the test solution (*B*). Arrows in histograms indicate mean amplitude of m.e.p.s.p.s. There was no detectable enkephalin-induced hyperpolarization in post-synaptic membrane.

for 25 min, the average quantal content returned to 1·8. On the other hand, the quantal size obtained from the ratio of the mean amplitude of the e.p.s.p.s and the average quantal content was 0·38 mV in control and 0·41 mV in the presence of enkephalin (Fig. 4, downward arrows on histograms). A similar decrease in the mean quantal content was obtained from two other neurones (1·2 and 1·7 in control to 0·8 and 1·1 in the presence of enkephalin, respectively) without significant change in quantal size. These results again suggest that enkephalin acts presynaptically to reduce ACh release.

Decreased frequency of miniature e.p.s.p.s. Miniature e.p.s.p.s (m.e.p.s.p.s) in this ganglion occurred at a low frequency. Tetanic preganglionic stimulation increased the frequency of the m.e.p.s.p.s in the majority of ganglion cells examined (see Martin & Pilar, 1964; Hunt & Nelson, 1965; Miledi & Thies, 1971). This increase in the frequency of the m.e.p.s.p.s is considered to be brought about by an increase in spontaneous release of transmitter due possibly to accumulation of calcium inside the preganglionic nerve terminal during tetanic stimulation (Beume & Pott, 1978). Amplitude histograms of the m.e.p.s.p.s following tetanic stimulations were obtained during a period of 60 s in control and in the presence of enkephalin (Fig. 5). The amplitude distribution of the control m.e.p.s.p.s (Fig. 5*A*) did not follow a normal

distribution curve. However, the frequency of m.e.p.s.p.s decreased from 197 to 88/min; this decrease in frequency occurred at every amplitude (Fig. 5*B*). The mean amplitude of these m.e.p.s.p.s was not significantly changed ($0{\cdot}54 \pm 0{\cdot}02$ mV in control and $0{\cdot}53 \pm 0{\cdot}03$ mV in the presence of enkephalin, as indicated by downward arrows in Fig. 5). Similar results could be obtained from two other neurones.

Less-common responses to enkephalin

Fig. 3*A* illustrates an experiment in which enkephalin inhibited the e.p.s.p.s and wash-out of enkephalin caused augmentation of the e.p.s.p.s. This was observed in a small population of cells ($n = 5$). In other cells, low concentrations of enkephalin (10 and 30 nM) caused augmentation of the e.p.s.p.s in three cells, inhibition of the e.p.s.p.s in one cell and no effect in six cells. In the same cells in which 100 nM reduced the e.p.s.p. amplitude, application of 10 or 30 nM increased the amplitude ($n = 2$). It was not possible to demonstrate any consistent effect of naloxone on the increase in e.p.s.p. amplitude induced by enkephalin.

DISCUSSION

The present study demonstrates that enkephalin (10 nM to 10 μM) inhibits transmission in the parasympathetic ciliary ganglion of the cat. This results both from a membrane hyperpolarization associated with an increase in potassium conductance, and from a presynaptic inhibition of transmitter release. Since a decrease of the parasympathetic output to the iris sphincter leads to mydriasis, the morphine-induced mydriasis of the cat might be explained by the enkephalin-induced reduction of transmission in the ciliary ganglion. On the other hand, the morphine-induced myosis in the dog is considered to be due mainly to the activation of pupillo-constricting neurones in the mid-brain (Lee & Wang, 1975). It was recently found that, in the rabbit, intravenous morphine caused a transient mydriasis followed by myosis and slow fluctuations of the pupillary size (Murray & Tallarida, 1982). It would be interesting to examine the effect of opiates and opioid peptides on neural transmission in the rabbit ciliary ganglion.

Both post-synaptic hyperpolarization and presynaptic inhibition of transmitter release are produced by enkephalin in myenteric neurones (North *et al.* 1979) and sympathetic inferior mesenteric ganglion cells (Konishi *et al.* 1979) of the guinea-pig. Morphine was also reported to reduce transmitter release (without obvious post-synaptic action) in guinea-pig sympathetic ganglia (Bornstein & Fields, 1979). However, enkephalin-containing fibres have been found to terminate mainly on soma and dendrites of sympathetic coeliac ganglion cells of the guinea-pig (Kondo & Yui, 1982). In the bull-frog sympathetic ganglion, studied with the sucrose-gap technique, enkephalin caused post-synaptic membrane hyperpolarization and inhibition of slow inhibitory post-synaptic potentials; morphine caused presynaptic membrane hyperpolarization (Wouters & Van den Bercken, 1979, 1980).

The enkephalin hyperpolarization of the cat ciliary ganglion cells was associated with an increased conductance, reversed in polarity at -90 mV and was not appreciably affected by changing sodium and chloride concentrations in the super-fusing solution. These observations indicate that the enkephalin-induced hyper-

polarization is due mainly to the activation of a potassium conductance, as in the myenteric neurones (Morita & North, 1982).

Slow current relaxations were often triggered by commanding pulses in the voltage-clamped ciliary ganglion cells, as shown in Fig. 2*A*. Slow inward relaxations of current were observed when hyperpolarizing command pulses exceeded −90 mV. Enkephalin had no detectable or consistent effect on the slow relaxations. The time course of the relaxations resembles that of the M-current relaxations in bull-frog sympathetic ganglion cells (Brown & Adams, 1980) but also that of inward relaxations which are found in guinea-pig hippocampal neurones and which are thought to reflect the passage of a novel current, I_Q (Halliwell & Adams, 1982). The nature of the slow relaxations in the ciliary ganglion cells of the cat remains to be resolved.

Several electrophysiological studies, including the present study, indicate that enkephalin may act at presynaptic sites to reduce ACh release (e.g. Konishi *et al.* 1979; Bixby & Spitzer, 1983). There is also biochemical and pharmacological evidence that enkephalin inhibits transmitter release in various tissues (see Beaumont & Hughes, 1979). However, the mechanisms underlying the inhibitory action of enkephalin on transmitter release have not been elucidated. The present experiments disclosed that enkephalin prevented the increase in the frequency of the m.e.p.s.p.s which is considered to be brought about by accumulation of intracellular calcium during repetitive spikes in preganglionic nerve terminals (Beume & Pott, 1978); enkephalin may act at presynaptic sites which control calcium availability for transmitter release. These findings are consistent with the observations on cultured dorsal root ganglion cells (Mudge, Leeman & Fischbach, 1979; Werz & Macdonald, 1982); enkephalin shortens calcium spikes apparently by modulating voltage-sensitive channels and this is thought to account for the inhibition of transmitter release. On the other hand, Szerb (1980) mentions that morphine does not act directly on the coupling system between calcium and transmitter release in the myenteric plexus, but reduces the population of active cells by membrane hyperpolarization. If the enkephalin-induced hyperpolarization which is associated with an increased membrane conductance occurs in the soma as well as in presynaptic fibres, a shunting of the membrane resistance or a shift of the membrane potential below the threshold or both may result in the blockade of spike invasion into the nerve terminals where transmitter is released (see Morita & North, 1981). In accordance with this, as described above, morphine causes presynaptic membrane hyperpolarization in frog ganglia (Wouters & Van den Bercken, 1980). If the propagation of spikes is blocked by the enkephalin hyperpolarization of a population of presynaptic fibres, the total amount of released ACh would be reduced. It is possible to speculate, therefore, that the enkephalin-induced reduction of transmitter release might be due both to inhibition of calcium entry and to hyperpolarization of the preganglionic nerve fibres.

Enkephalin occasionally showed an augmentative or biphasic action on the e.p.s.p.s. This is interesting because enkephalin has been suggested to cause presynaptic facilitation of the e.p.s.p.s in the rat hippocampus (Haas & Ryall, 1980; but see Masukawa & Prince, 1982). It was also found that opiates prolong the calcium-dependent after-hyperpolarizations of myenteric neurones at concentrations (100 pM–10 nM) which are considerably lower than those usually required to hyperpolarize the resting membrane (Tokimasa, Morita & North, 1981). Furthermore,

morphine has been reported to produce dual actions on calcium-dependent responses of nodose ganglion cells, such as the calcium spike, the shoulder of the action potential and the hyperpolarizing after-potential (Higashi, Shinnick-Gallagher & Gallagher, 1982). These authors stated that there is a tendency for augmentation of the calcium-dependent spikes at lower concentrations (1 nM). These observations may be consistent with the dual action of enkephalin on the e.p.s.p.s in the ciliary ganglion of the cat.

We wish to thank Dr R. Alan North for his help and criticism in the preparation of the manuscript and Miss Michiko Sakai for typing the manuscript.

REFERENCES

BEAUMONT, A. & HUGHES, J. (1979). Biology of opioid peptides. *A. Rev. Pharmac. Toxicol.* **19**, 245–267.

BEUME, R. & POTT, L. (1978). Dual effect of external calcium on the frequency of miniature synaptic potentials in frog sympathetic ganglion cells. *Pflügers Arch.* **376**, 21–26.

BIXBY, J. L. & SPITZER, N. C. (1983). Enkephalin reduces quantal content at the frog neuromuscular junction. *Nature, Lond.* **301**, 431–432.

BORNSTEIN, J. C. & FIELDS, H. L. (1979). Morphine presynaptically inhibits a ganglionic cholinergic synapse. *Neurosci. Lett.* **15**, 77–82.

BROWN, D. A. & ADAMS, P. R. (1980). Muscarinic suppression of a novel voltage-sensitive K^+ current in a vertebrate neurone. *Nature, Lond.* **283**, 673–676.

ERICHSEN, J. T., REINER, A. & KARTEN, H. J. (1982). Co-occurrence of substance P-like and leu-enkephalin-like immunoreactivities in neurones and fibres of avian nervous system. *Nature, Lond.* **295**, 407–410.

GINSBORG, B. L. & KADO, R. T. (1975). Voltage–current relationship of a carbachol-induced potassium-ion pathway in *Aplysia* neurones. *J. Physiol.* **245**, 713–725.

GRIFFITH, W. H., GALLAGHER, J. P. & SHINNICK-GALLAGHER, P. (1980). An intracellular investigation of cat vesical pelvic ganglia. *J. Neurophysiol.* **43**, 343–354.

HAAS, H. L. & RYALL, R. W. (1980). Is excitation by enkephalins of hippocampal neurones in the rat due to presynaptic facilitation or to disinhibition? *J. Physiol.* **308**, 315–330.

HALLIWELL, J. V. & ADAMS, P. R. (1982). Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. *Brain Res.* **250**, 71–92.

HIGASHI, H., SHINNICK-GALLAGHER, P. & GALLAGHER, J. P. (1982). Morphine enhances and depresses Ca^{2+}-dependent responses in visceral primary afferent neurons. *Brain Res.* **251**, 186–191.

HUGHES, J., SMITH, T. W., KOSTERLITZ, H. W., FOTHERGILL, L. A., MORGAN, B. A. & MORRIS, H. R. (1975). Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature, Lond.* **258**, 577–579.

HUNT, C. C. & NELSON, P. G. (1965). Structural and functional changes in the frog sympathetic ganglion following cutting of the presynaptic nerve fibres. *J. Physiol.* **177**, 1–20.

JOHNSON, D. A. & PURVES, D. (1981). Post-natal reduction of neural unit size in the rabbit ciliary ganglion, *J. Physiol.* **318**, 143–159.

KATAYAMA, Y. & NISHI, S. (1981). Actions of enkephalin on single neurons in ciliary ganglia. In *Advances in Endogenous and Exogenous Opioids*, ed. TAKAGI, H. & SIMON, E., pp. 205–207. Tokyo: Kodansha.

KATAYAMA, Y. & NISHI, S. (1982). Voltage-clamp analysis of peptidergic slow depolarizations in bullfrog sympathetic ganglion cells. *J. Physiol.* **333**, 305–313.

KONDO, H. & YUI, R. (1982). An electron microscopic study on enkephalin-like immunoreactive nerve fibers in the celiac ganglion of guinea-pigs. *Brain Res.* **252**, 142–145.

KONISHI, S., TSUNOO, A. & OTSUKA, M. (1979). Enkephalins presynaptically inhibit cholinergic transmission in sympathetic ganglia. *Nature, Lond.* **282**, 515–516.

LEE, H. K. & WANG, S. C. (1975). Mechanism of morphine-induced miosis in the dog. *J. Pharmac. exp. Ther.* **192**, 415–431.

MARTIN, A. R. & PILAR, G. (1964). Presynaptic and post-synaptic events during post-tetanic potentiation and facilitation in the avian ciliary ganglion. *J. Physiol.* **175**, 17–30.

MASUKAWA, L. M. & PRINCE, D. A. (1982). Enkephalin inhibition of inhibitory input to CA1 and CA3 pyramidal neurons in the hippocampus. *Brain Res.* **249**, 271–280.

MILEDI, R. & THIES, R. (1971). Tetanic and post-tetanic rise in frequency of miniature end-plate potentials in low-calcium solutions. *J. Physiol.* **212**, 245–257.

MORITA, K. & NORTH, R. A. (1981). Opiates and enkephalin reduce the excitability of neuronal processes. *Neuroscience* **6**, 1943–1951.

MORITA, K. & NORTH, R. A. (1982). Opiate activation of potassium conductance in myenteric neurones: inhibition by calcium ion. *Brain Res.* **242**, 145–150.

MUDGE, A. W., LEEMAN, S. E. & FISCHBACH, G. D. (1979). Enkephalin inhibits release of substance P from sensory neurons in culture and decreases action potential duration. *Proc. natn. Acad. Sci. U.S.A.* **76**, 526–530.

MURRAY, R. B. & TALLARIDA, R. J. (1982). Pupillographic analysis of morphine action in the rabbit: role of the autonomic nervous system. *Eur. J. Pharmacol.* **80**, 197–202.

NISHI, S. & CHRIST, D. D. (1971). Electrophysiological properties and activities of mammalian parasympathetic ganglion cells. *Fedn Proc.* **30**, 489.

NORTH, R. A., KATAYAMA, Y. & WILLIAMS, J. T. (1979). On the mechanism and site of action of enkephalin on single myenteric neurons. *Brain Res.* **165**, 67–77.

NORTH, R. A. & TONINI, M. (1977). The mechanism of action of narcotic analgesics in the guinea-pig ileum. *Br. J. Pharmac.* **61**, 541–549.

PEPPER, C. M. & HENDERSON, G. (1980). Opiates and opioid peptides hyperpolarize locus coeruleus neurons in vitro. *Science, N.Y.* **209**, 394–396.

PERRY, W. L. M. & TALESNIK, J. (1953). The role of acetylcholine in synaptic transmission at parasympathetic ganglia. *J. Physiol.* **119**, 455–469

SUZUKI, T. & VOLLE, R. L. (1979). Nicotinic, muscarinic and adrenergic receptors in a parasympathetic ganglion. *J. Pharmac. exp. Ther.* **211**, 252–256.

SZERB, J. C. (1980). Effect of low calcium and of oxotremorine on the kinetics of the evoked release of [^{3}H]acetylcholine from the guinea-pig myenteric plexus; comparison with morphine. *Naunyn-Schmiedebergs Arch. Pharmacol.* **311**, 119–127.

TOKIMASA, T., MORITA, K. & NORTH, R. A. (1981). Opiates and clonidine prolong calcium-dependent after-hyperpolarizations. *Nature, Lond.* **294**, 162–163.

WERZ, M. A. & MACDONALD, R. L. (1982). Heterogenous sensitivity of cultured dorsal root ganglion neurones to opioid peptides selective for μ- and δ-opiate receptors. *Nature, Lond.* **299**, 730–733.

WILLIAMS, J. T., EGAN, T. M. & NORTH, R. A. (1982). Enkephalin opens potassium channels on mammalian central neurones. *Nature, Lond.* **299**, 74–77.

WOUTERS, W. & VAN DEN BERCKEN, J. (1979). Hyperpolarisation and depression of slow synaptic inhibition by enkephalin in frog sympathetic ganglion. *Nature, Lond.* **277**, 53–54.

WOUTERS, W. & VAN DEN BERCKEN, J. (1980). Effects of met-enkephalin on slow synaptic inhibition in frog sympathetic ganglion. *Neuropharmacology* **19**, 237–243.