EFFECTS OF ACTH$_{4-10}$ ON SYNAPTIC TRANSMISSION IN FROG SYMPATHETIC GANGLION

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The influence of ACTH$_{4-10}$, a behaviourally active fragment of adrenocorticotropic hormone (ACTH) devoid of endocrine activity, on synaptic transmission in the paravertebral sympathetic ganglion of the frog was investigated. Postsynaptic potentials evoked by electrical stimulation of preganglionic nerves were recorded using a sucrose gap method. Fast excitatory postsynaptic potentials (EPSPs), which are mediated via nicotinic cholinergic synapses, were not affected by 10^{-6} M ACTH$_{4-10}$. Application of ACTH$_{4-10}$ in a concentration as low as 10^{-8} M for 60 min caused a marked augmentation of the amplitude of slow inhibitory postsynaptic potentials (IPSPs) which are mediated via dopaminergic synapses. The increase in amplitude developed gradually after a latency of 60—90 min and outlasted the application of the peptide. In addition, ACTH$_{4-10}$ at 10^{-6} M increased the hyperpolarising response of the ganglion to exogenous dopamine, as studied by a micro-application method. There was no significant effect of ACTH$_{4-10}$ on the muscarinic cholinergic depolarising response of the ganglion towards exogenous acetylcholine. The behaviourally active vasopressin fragment DG-LVP (10^{-6} M) had no effect on slow IPSPs. The results demonstrate that ACTH$_{4-10}$ specifically affects slow synaptic inhibition in frog sympathetic ganglion, probably by acting upon the postsynaptic membrane. The possibility is discussed that ACTH$_{4-10}$ affects one of the intermediate steps between dopaminergic receptor interaction and generation of the slow IPSP.

1. Introduction

There is considerable evidence that the adrenocorticotropic hormone (ACTH) influences learned behaviour by acting directly on the central nervous system (De Wied, 1974, 1977; Kastin et al., 1977; Gispen et al., 1977). ACTH has been shown to restore performance completely in hypophysectomised rats which show impaired learning of a conditioned response. In normal rats ACTH delays the extinction of active and passive avoidance behaviour. ACTH and the closely related melanocyte stimulating hormone (MSH) have many behavioural effects in common. The neurohypophyseal hormone vasopressin is also known to affect avoidance behaviour (De Wied et al., 1976).

The behavioural effects of these hormones are not due to their endocrine actions, for fragments of these peptides such as ACTH$_{4-10}$ and DG-LVP which are practically devoid of endocrine activity produce very similar effects (De Wied, 1967; De Wied et al., 1972). ACTH$_{4-10}$ has also been shown to affect visual attention in healthy humans (Kastin et al., 1971; Miller et al., 1974).

ACTH and related peptides have been found to affect electrophysiological parameters of the central nervous system. In rats (Sandman et al., 1971) and frogs (Denman et al., 1972) α- and β-MSH augmented the 4—9 Hz EEG activity. ACTH$_{4-10}$ lowered the content of the 3—7 Hz band of the EEG of humans and increased the frequency content above 7 Hz (Miller et al., 1974). The

dominant frequency in the electrically evoked hippocampal theta rhythm of the rat was found to shift to a higher frequency after administration of $ACTH_{4-10}$. Since this effect could be mimicked by increasing the stimulus strength, it was thought to be indicative of an excitatory action of $ACTH_{4-10}$ (Urban and De Wied, 1976; 1978). Neurons in the hypothalamic and mesoencephalic area of the rat were activated by iontophoretic application of ACTH (Steiner, 1970). Systemic administration of ACTH increased the single unit activity in the hypothalamus of the rat (Van Delft and Kitay, 1972) and decreased the multiple unit activity in the medial preoptic area of the cat (Korányi et al., 1971). It has been reported that β-MSH, but not $ACTH_{1-24}$, enhanced reflex activity in the spinal cord of the cat. Both peptides antagonized the depressive action of morphine on ventral root potentials in the cat and in the frog (Zimmerman and Krivoy, 1973; 1974).

ACTH and related peptides have also been shown to affect the peripheral nervous system. Torda and Wolff (1952) found the amplitude of the compound muscle action potential of the m.gastrocnemius in situ to be greatly reduced in hypophysectomized rats. Systemic application of ACTH, but not of corticosteroids, restored the action potential amplitude. Using the same preparation in hypophysectomized rats, Strand et al., (1973/74) and Strand and Cayer (1975) observed that muscle fatigue produced by prolonged nerve stimulation was decreased after administration of ACTH, α-MSH, β-MSH and $ACTH_{4-10}$. In squid giant axon membrane ACTH did not affect the sodium and potassium currents underlying the action potential (Henkin et al., 1973).

Although the physiological mechanisms by which ACTH and related neuropeptides affect the nervous system are not known, it is generally assumed that they act as modulators of synaptic transmission. It is not yet clear which type of synapses and which neurotransmitters are involved in the neurogenic actions of these peptides. Torda

and Wolff (1952) have demonstrated an increase in acetylcholine synthesis in rat brain after ACTH administration. In recent years more attention has been paid to catecholaminergic systems. Stressful stimuli or treatment with $ACTH_{4-10}$ have been shown to increase noradrenaline turnover in the brain of normal rats, while it is decreased by hypophysectomy (Versteeg and Wurtman, 1976). Dopamine turnover in rat brain was also increased after treatment with ACTH fragments (Iuvone et al., 1978).

The present study was undertaken in order to obtain further information about the influence of $ACTH_{4-10}$ on synaptic transmission. The paravertebral sympathetic ganglion of the frog was used as a model. The effect of DG-LVP was also tested in one series of experiments. The sympathetic ganglion is particularly well-suited for such an investigation. It manifests both fast and slow synaptic activity, mediated via different, well-defined, synaptic pathways which can be studied independently. Fig. 1 shows a schematic
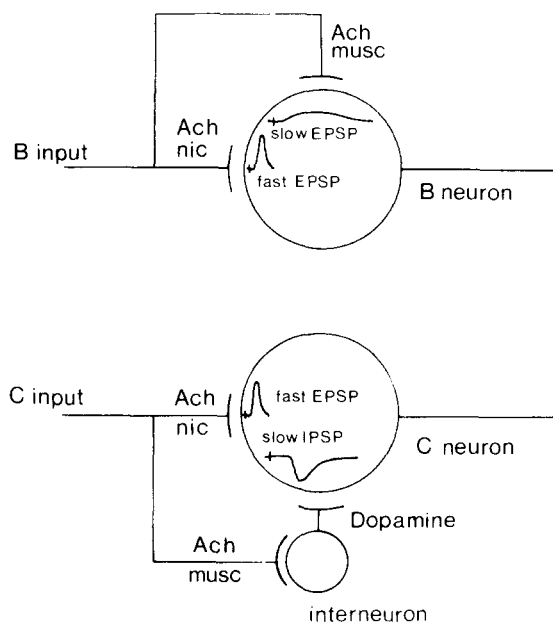

Fig. 1. Schematic diagram of synaptic pathways in the ninth or tenth paravertebral sympathetic ganglion of the frog. nic = nicotinic, musc = muscarinic (after Kalix et al., 1974 and Libet, 1970).

diagram of the synaptic pathways within the ninth or tenth vertebrate sympathetic ganglion, which have the same functional anatomy. More detailed information can be found elsewhere (Skok, 1973; Nishi, 1976). The ganglia contain two different types of neurons, classified as B and C neurons respectively. Postsynaptic potentials can be evoked by stimulation of the preganglionic nerves. Fast excitatory postsynaptic potentials (EPSPs) are generated in B and C neurons via a nicotinic cholinergic synapse. Slow EPSPs are generated in B neurons via a muscarinic cholinergic synapse. Slow inhibitory postsynaptic potentials (IPSPs) occur in C neurons and are brought about by a catecholamine, probably dopamine (Libet, 1970; Libet and Tasaka, 1970) released from an interneuron which in turn is activated via a muscarinic cholinergic synapse.

The results show that ACTH$_{4-10}$ can affect dopaminergic synaptic transmission. After a delay of 60–90 min ACTH$_{4-10}$ in a concentration of 10^{-8} M caused a persistent potentiation of slow synaptic inhibition, but did not affect fast excitatory synaptic transmission. Further experimentation has revealed that the effects of ACTH$_{4-10}$ may be attributed to a postsynaptic mechanism.

2. Materials and methods

2.1. Preparation

The experiments were carried out on the paravertebral sympathetic ganglion of the frog (*Rana esculenta* and *R. temporaria*). The frogs were obtained from a local supplier and were kept in the laboratory at a temperature of about 20°C. They were fed regularly. The animals were gently taken out of the tank and decapitated immediately to avoid stress-induced release of ACTH, and were then pithed. The caudal portion of the paravertebral sympathetic chain including the ganglia six to ten was dissected together with the rami communicantes and parts of the spinal

nerves. The ganglia were freed of excess connective tissue to permit better penetration by the drugs. Either the ninth or the tenth sympathetic ganglion could be used throughout this study because they have the same functional anatomy. The experiments were performed at a temperature of 21–23°C.

2.2. Recording and stimulation

Ganglionic potentials were recorded using the sucrose gap method (Nishi and Koketsu, 1968; Wallis et al., 1975). The preparation was mounted in a Perspex chamber across two 0.3 mm wide partitions separated by a 0.4 mm sucrose gap. The postganglionic nerve of the ninth or tenth sympathetic ganglion was placed in a small groove in the partitions and was sealed with silicone grease. The ganglionic potentials were led off by a pair of Ag/AgCl electrodes, fed into a d.c.-coupled amplifier with automatic drift compensation and displayed on an oscilloscope. The d.c. potential across the sucrose gap was recorded on a strip chart recorder. The electrically evoked postganglionic potentials were digitized with the aid of a transient recorder (Biomation, model 802), averaged on computer and plotted. Much care was taken to ensure stable recording throughout the experiment. In most experiments d.c. stability was better than 1 mV per h. The resistance across the sucrose gap was monitored continuously. Results of experiments in which this resistance was not stable were discounted.

Fast EPSPs were evoked by stimulating the paravertebral chain at the level of the seventh ganglion (B input; see fig. 1) by supramaximum pulses lasting 0.1 msec delivered through a suction electrode. Every 20 min 5 trains of 20 fast EPSPs (40 Hz) were elicited at a rate of 1 train min^{-1} and these were averaged. In order to avoid generation of postganglionic action potentials, the fast EPSPs were partially blocked by 10^{-4} M hexamethonium chloride (Lees and Nishi, 1972).

Slow IPSPs were elicited by stimulating the ramus communicans to the eighth ganglion (C

input; see fig. 1) by a second suction electrode with a train of 5 pulses (0.1 msec; 10 Hz). The simultaneously evoked fast EPSPs were blocked selectively by the continuous presence of $3 \cdot 10^{-5}$ M nicotine sulphate. Every 30 min 5 slow IPSPs were evoked at a rate of 1 slow IPSP min^{-1} and these were averaged.

2.3. Chemically induced ganglionic potentials

The effect of ACTH$_{4-10}$ on the chemical sensitivity of the ganglion to exogenous dopamine or to acetylcholine was tested in the following way. The tip of a 27 gauge injection needle was positioned just over the ganglion with the aid of a micromanipulator. The needle was connected to a 1 ml syringe which was driven by an electronically controlled step motor. In this way small, discrete, amounts (approximately 1 μl) of a 0.1 M solution of the transmitter in Ringer were ejected at intervals which produced transient responses with a constant amplitude in control experiments. In these experiments $3 \cdot 10^{-5}$ M nicotine sulphate was continuously present in the perfusion solution. Ejection of Ringer alone was without effect. The chemically induced potentials were recorded on paper.

2.4. Solutions and drugs

The ganglion was continuously superfused at a rate of 0.5 ml min^{-1} (bath volume 0.2 ml) with Ringer solution containing in mM: NaCl 116, KCl 2.4, CaCl$_2$ 1.8 and HEPES buffer 3; pH 7.3. The postganglionic nerve was superfused with isotonic KCl (119 mM).

Drugs were applied to the ganglion by switching the superfusion from the normal Ringer solution to one which contained the desired concentration of the drugs. Hexamethonium chloride (Fluka AG), nicotine sulphate (Sigma), dopamine hydrochloride (Fluka AG) and acetylcholine chloride (BDH) were dissolved in Ringer to make up stock solutions. ACTH$_{4-10}$ and DG-LVP (kindly sup-

plied by Prof. Dr. D. De Wied, Rudolf Magnus Institute for Pharmacology, Utrecht) were dissolved in HCl Ringer at pH 4. Shortly before the experiments the stock solutions were diluted with Ringer at pH 7.3 to obtain the final drug concentration.

3. Results

3.1. Resting membrane potential

Upon the application of ACTH$_{4-10}$ or DG-LVP (10^{-6} M) the d.c. potential across the sucrose gap was carefully monitored to see whether these peptides exerted an effect on the resting membrane potential of the ganglionic neurons. Although the stability of the recording method permitted the observation
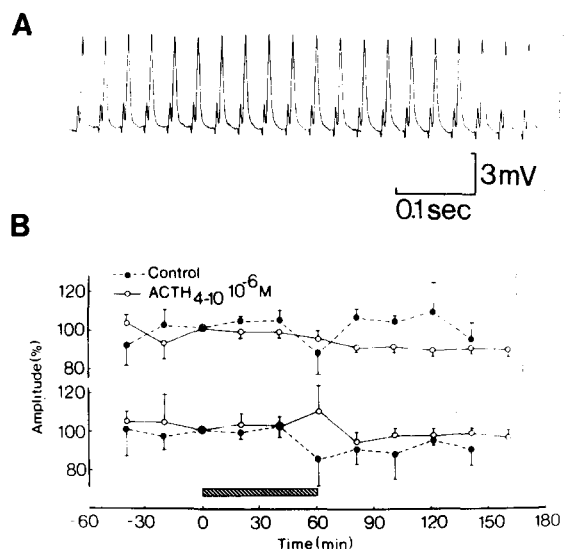

Fig. 2. (A) Train of fast EPSPs in a control experiment. (B) Upper curves: Amplitude of first fast EPSP. Lower curves: Mean amplitude of last 10 fast EPSPs. 10^{-6} M ACTH$_{4-10}$ was applied for 60 min starting at time zero as indicated by horizontal bar. Five trains of fast EPSPs (40 Hz; 0.5 sec) were evoked every 20 min and were averaged. Hexamethonium chloride (10^{-4} M) was continuously present in the superfusion solution to prevent generation of postsynaptic action potentials. Amplitudes are expressed as a percentage of amplitude at time zero. Each point represents the mean ± S.D. of 3 ACTH$_{4-10}$ or 3 control experiments.

of slight changes in the d.c. potential, no effect was observed in any of the experiments.

3.2. Fast excitatory postsynaptic potentials

The influence of ACTH$_{4-10}$ on fast EPSPs was studied in preparations that were stimulated with short trains of pulses (0.5 sec; 40 Hz; see fig. 2A). Such a stimulation procedure puts a certain load on the synaptic system; a condition that may reveal the effects of ACTH$_{4-10}$ more clearly. The amplitude of the first fast EPSP of each train was measured together with the mean amplitude of the last 10 fast EPSPs of each train. In control experiments both parameters remained constant over a period of hours (fig. 2B). Treatment with 10^{-6} M ACTH$_{4-10}$ for 60 min did not produce a significant change in the amplitude of the first fast EPSP or in the mean amplitude of the last 10 fast EPSPs of the train over a period of more than 2 h (fig. 2B). Duration and time course of the fast EPSPs were not affected by ACTH$_{4-10}$. Higher concentrations of ACTH$_{4-10}$ or higher stimulus frequencies were not tested.

3.3. Potentiation of slow IPSP by ACTH$_{4-10}$

In control experiments 5 slow IPSPs were evoked every 30 min at a rate of 1 min^{-1}. Though much care was taken to achieve a stable recording, the amplitude of the slow IPSP gradually declined over a period of 4.5 h (see fig. 4). This probably reflects the non-optimum conditions of the in vitro experiments.

After treatment with 10^{-8} M ACTH$_{4-10}$ for 60 min the amplitude of the slow IPSP no longer declined. Instead, the slow IPSP started to increase gradually and eventually attained an amplitude considerably higher than that at the beginning of ACTH application. Fig. 3 shows averaged slow IPSPs before and at different times after the application of 10^{-8} M ACTH$_{4-10}$. The results of a series of 5 experiments together with the control curve are depicted in fig. 4. The increase in slow
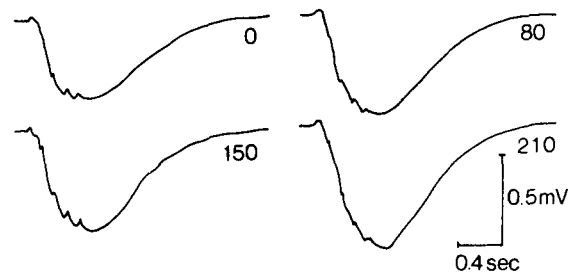

Fig. 3. Slow IPSPs before and at intervals after treatment with ACTH$_{4-10}$. 10^{-8} M ACTH$_{4-10}$ was present between time 0 and time 60 min. Numbers indicate time after start of ACTH treatment in minutes. Each tracing is the averaged result of 5 slow IPSPs evoked by repetitive stimulation (0.5 sec; 10 Hz) at a rate of 1 slow IPSP per min. Nicotine sulphate $3 \cdot 10^{-5}$ M was continuously present in the superfusion solution so as to suppress the simultaneously evoked fast EPSPs. The slow IPSP amplitude at time 210 min was 161% of the amplitude at time zero. Deflections during first part of slow IPSPs were due to stimulus artefacts and to incompletely suppressed fast EPSPs.

IPSP amplitude became apparent between 60 and 90 min after the application of ACTH$_{4-10}$ was started and it continued till the end of the experiments, namely 150 min after the peptide treatment was stopped. At this time the mean amplitude of the slow IPSP amounted to 130 ± 24% (mean ± S.D.; n = 5)
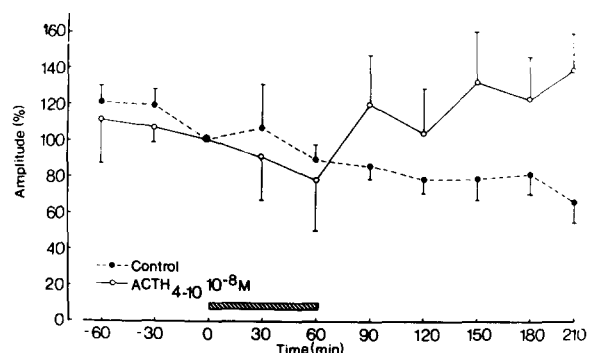

Fig. 4. Augmentation of slow IPSP amplitude by ACTH$_{4-10}$. 10^{-8} M ACTH$_{4-10}$ was applied for 60 min starting at time zero as indicated by horizontal bar. Amplitudes of slow IPSPs are expressed as a percentage of the amplitude at time zero. Each point represents the mean ± S.D. of 5 ACTH or 3 control experiments.

of the amplitude at the beginning of the ACTH treatment. Compared to the amplitude of the slow IPSP in the control experiments at time 210 min, the amplitude of the slow IPSP in the ACTH experiments reached a value of $172 \pm 27\%$.

Linear regression analysis was used to compare the data from the ACTH experiments with those from the control experiments. The control data from time -60 min to time 210 min could be fitted by one straight line. The data from the ACTH-treated ganglia could be fitted by two straight lines: one line from time -60 min to time 60 min, which did not differ significantly from the control line in this period $(P = 0.14)$, and a second line from time 60 min to time 210 min. This last line differed from the line for the control data $(P = 0.004)$.

To see whether $ACTH_{4-10}$ affected the duration of the slow IPSP, the areas of the averaged slow IPSPs were measured. It appeared that the percentage increase in area after the application of $ACTH_{4-10}$ was always equivalent to the percentage increase in amplitude. This indicates that the duration of the slow IPSP was not greatly affected by $ACTH_{4-10}$.

A concentration of 10^{-6} M $ACTH_{4-10}$ also caused an increase in amplitude of the slow IPSP similar to that observed after 10^{-8} M (4 expts.). Likewise, the amplitude of the slow IPSP did not start to increase until 60–90 min after the application of the peptide. Since a concentration of 10^{-8} M appeared to produce a maximum effect, a concentration of 10^{-10} M was also tested, but had no clear effect.

Desglycinamide-lysine-vasopressine (DG-LVP) was also tested on slow synaptic inhibition under the same experimental conditions. Application of 10^{-6} M DG-LVP for 60 min did not have a significant effect on amplitude (fig. 5) or on duration of the slow IPSP. Linear regression analysis showed that the data could be fitted by two straight lines, one for the control data and one for the DG-LVP experiments, which did not differ signifi-
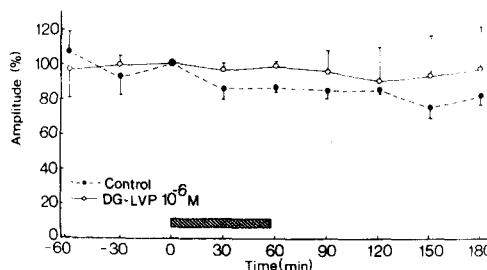

Fig. 5. Absence of effect of DG-LVP on amplitude of slow IPSP. 10^{-6} M DG-LVP was present for 60 min starting at time zero as indicated by horizontal bar. Amplitudes of slow IPSPs are expressed as a percentage of amplitude at time zero. Each point represents the mean $\pm$ S.D. of 5 DG-LVP or 2 control experiments.

cantly from each other $(P = 0.08)$ or from the control line in fig. 4 $(P = 0.16)$.

3.4. Increase in dopamine-induced hyperpolarisations by $ACTH_{4-10}$

The slow IPSP in the vertebrate sympathetic ganglion is considered to be brought about by the release of dopamine from an interneuron, which in its turn is activated via a muscarinic cholinergic synapse (see fig. 1). The potentiation of the slow IPSP by $ACTH_{4-10}$ described in the previous section could be due to at least two different mechanisms, either to an increase in the amount of dopamine released from the presynaptic nerve terminals, or to an enhanced sensitivity of the postsynaptic neuronal membrane to the transmitter. In an attempt to discriminate between these two possibilities, the influence of $ACTH_{4-10}$ on the sensitivity of the ganglion to exogenous dopamine was studied by means of the micro-ejection method. A single ejection of dopamine (about 1 μl of a 0.1 M solution) produced a transient hyperpolarisation of the ganglionic neurons with a time course of several minutes and with an amplitude of 0.5–3 mV in various experiments (fig. 6). If the ejections were repeated at intervals of 10 min in control experiments a reproducible response could be obtained; both amplitude
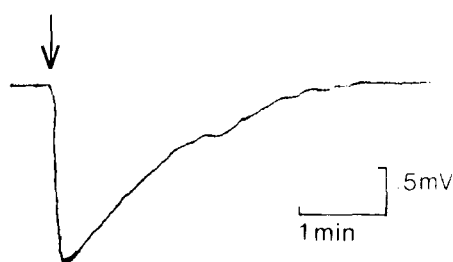
Fig. 6. Transient hyperpolarisation produced by micro-application of dopamine. At the moment indicated by the arrow, 1 μl of a 0.1 M dopamine solution in Ringer was applied just over the ganglion.

and duration of the dopamine-induced hyperpolarisations remained virtually constant over a period of several hours. This is illustrated in fig. 7 in which the area of the hyperpolarisations is plotted against time.

Application of ACTH$_{4-10}$ in a concentration of 10^{-6} M for 60 min caused a gradual increase in the dopamine-induced hyperpolarisations (fig. 7). This increase developed slowly and continued till the end of the experiments, thus outlasting the presence of the peptide in the perfusion solution. The area of the hyperpolarisations increased more than the amplitude. This may indicate that
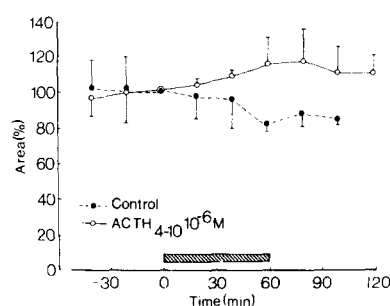

Fig. 7. Effect of ACTH$_{4-10}$ on dopamine-induced hyperpolarisations. 10^{-6} M ACTH$_{4-10}$ was applied for 60 min starting at time zero as indicated by horizontal bar. Hyperpolarisations were evoked at a rate of 1 per 10 min and 2 successive responses were averaged. Areas of hyperpolarisations are expressed as a percentage of area at time zero. Each point represents the mean $\pm$ S.D. of 4 ACTH$_{4-10}$ or 3 control experiments.

ACTH$_{4-10}$ not only increased the amplitude, but also the duration of the response. It should be noted, however, that the duration of responses evoked by external application of a transmitter cannot be assessed adequately because of the prolonged presence of an excess of transmitter. Linear regression analysis showed that the line for the control data differed significantly (P = 0.02) from the line for the ACTH experiments.

3.5. Acetylcholine-induced potentials

The slow EPSP in the vertebrate sympathetic ganglion is mediated via muscarinic cholinergic synapses (see fig. 1). Slow EPSPs were elicited by repetitive stimulation of the preganglionic B nerve in preparations in which the fast EPSPs were blocked selectively by the continuous presence of $3 \cdot 10^{-5}$ M nicotine. Their amplitude was small, usually less than 0.2 mV, and they had a duration of 10—20 sec. Both amplitude and duration of the slow EPSP varied considerably during the course of an experiment. In some preparations the presence of a slow EPSP could not be clearly demonstrated (cf. Libet et al., 1968). Therefore, the effect of ACTH$_{4-10}$ on muscarinic cholinergic sensitivity of the ganglion was studied by micro-application of exogenous acetylcholine in nicotine-blocked ganglia. A single ejection of 1 μl of 0.1 M acetylcholine produced a biphasic response: a hyperpolarisation followed by a long-lasting depolarisation. The hyperpolarisation probably results from activation of the interneurons and is related to the slow IPSP. The depolarisation results from activation of the muscarinic receptors on B neurons and is analogous to the slow EPSP (Libet, 1970). Because of their small amplitude (0.5—1 mV) and long duration (10—15 min) the acetylcholine-induced depolarisations were difficult to measure accurately. Exposure of the ganglion to 10^{-6} M ACTH$_{4-10}$ produced no consistent effects. In some but not all, preparations the amplitude of the slow depolarisations tended to increase.

4. Discussion

The present results show that $ACTH_{4-10}$ in a concentration as low as 10^{-8} M induced a potentiation of slow synaptic inhibition in frog sympathetic ganglion. This is the first electrophysiological demonstration that $ACTH_{4-10}$ has a direct effect on synaptic transmission. The effect of $ACTH_{4-10}$ seems to be a specific one and cannot be attributed to a general improvement of synaptic transmission as a result of peptide treatment. Application of DG-LVP under identical experimental conditions had no significant effect, and $ACTH_{4-10}$ in a concentration of 10^{-6} M failed to affect fast excitatory synaptic transmission. Further, the pentapeptide met-enkephalin was found to cause a depression of slow synaptic inhibition in frog sympathetic ganglion (Wouters and Van den Bercken, 1979).

In addition to causing an augmentation of slow IPSP amplitude, $ACTH_{4-10}$ in a concentration of 10^{-6} M caused an increase in dopamine-induced hyperpolarisation of the ganglionic neurons. Comparison of both phenomena (figs. 4 and 7) shows that their time course and magnitude were very similar. This strongly suggests that the same mechanism is responsible for the potentiation of slow synaptic inhibition and the augmentation of the dopamine-induced hyperpolarisations. The most likely explanation is that in both cases $ACTH_{4-10}$ enhances the sensitivity of the postsynaptic neuronal membrane to dopamine.

Typical characteristics of the $ACTH_{4-10}$-induced effect reported here are its long latency and its long duration. This has also been found in other studies. In behavioural experiments with rats the effect of a single injection of $ACTH_{4-10}$ lasts for several hours (De Wied, 1974). The shift in dominant frequency of the electrically evoked hippocampal theta activity induced by $ACTH_{4-10}$ in the rat reached its maximum 1–2 h after subcutaneous peptide application, and lasted for several hours (Urban and De Wied, 1976).

$ACTH_{4-10}$ has a remarkably long-lasting effect: in the present experiments the effect outlasted the application of the peptide by more than 2 h. This suggests that $ACTH_{4-10}$ triggers some mechanism which, once started, no longer requires the presence of the peptide. The possibility that a fraction of $ACTH_{4-10}$ is still bound within the ganglion can, however, not be excluded. It is not unlikely that $ACTH_{4-10}$ affects one of the steps between postsynaptic receptor activation by dopamine and generation of the slow IPSP. There is evidence that the activation of dopaminergic receptors involved in the slow synaptic inhibition in mammalian sympathetic ganglion results in the formation of cyclic AMP by virtue of activation of a dopamine sensitive adenylate cyclase. This cyclic AMP is postulated to regulate the activity of a protein kinase, which in turn controls the level of phosphorylation of specific membrane proteins and thereby electrogenesis in the postsynaptic membrane (Kalix et al., 1974; Greengard, 1976; Nathanson, 1977).

At present no conclusive data on the effects of ACTH on these processes are available. It has been reported that short-term incubation with ACTH did not affect the formation of cyclic AMP in mammalian brain slices (Forn and Krishna, 1971) or the activity of adenylate cyclase in rat brain broken cell preparation (Von Hungen and Roberts, 1973). Recently, however, Wiegant et al. (1979), have reported an increase in cyclic AMP levels in rat brain slices incubated with 10^{-5} M $ACTH_{1-24}$. In concentrations below 2.5×10^{-5} M this peptide also induced a weak stimulation of adenylate cyclase activity in rat brain broken cell preparations, whereas higher concentrations caused an inhibition. Such an inhibition was also caused by $ACTH_{4-7}$ and $ACTH_{1-16}\text{-}NH_2$, but not by other sequences of ACTH, including $ACTH_{1-10}$ and $ATCH_{4-10}$. Another possibility is that ACTH modulates the activity of phosphodiesterase which is responsible for the breakdown of cyclic AMP. This is an interesting possibility because a variety of substances

which inhibit phosphodiesterase activity, including the methylxanthines, are also known to have behavioural effects (Nathanson, 1977). Membrane phosphorylation may also be affected by ACTH. Zwiers et al., (1976, 1978) reported that 10^{-4} M ACTH$_{1-24}$ decreased the phosphorylation of distinct membrane proteins in vitro. ACTH$_{1-16}$ was as effective as ACTH$_{1-24}$, but the shorter sequences ACTH$_{1-10}$ and ACTH$_{4-10}$ were ineffective.

The dopaminergic interneurons are innervated by muscarinic cholinergic fibres, so that ACTH$_{4-10}$ could affect this type of synapses thereby producing the potentiation of the slow IPSP. This possibility seems unlikely however since it was found that ACTH$_{4-10}$ also caused an increase in the hyperpolarisation produced by exogenous dopamine. Further, no evidence was found for an effect of ACTH$_{4-10}$ on the slow EPSP which is mediated via muscarinic cholinergic synapses, or on the muscarinic cholinergic response of the ganglion to exogenous acetylcholine.

In the present study, ACTH$_{4-10}$ was not found to affect fast excitatory synaptic transmission, which is mediated via nicotinic cholinergic synapses. Neither the amplitude of the first fast EPSP in a train nor the maintenance of the fast EPSP amplitude during the train were influenced by 10^{-6} M ACTH$_{4-10}$. The effects of ACTH on nicotinic cholinergic transmission have been studied previously in an in situ nerve—muscle preparation of the rat (Torda and Wolff, 1952; Strand et al., 1973/74; Strand and Cayer, 1975). These authors have reported a restorative effect of ACTH and ACTH$_{4-10}$ on the amplitude of the muscle action potential during repetitive nerve stimulation. It could be that in the present experiments the frequency or duration of the repetitive stimulation was not sufficient to demonstrate a possible restorative effect of ACTH$_{4-10}$ on the fast EPSP. However, application of 10^{-6} M ACTH$_{4-10}$ did not affect the amplitude of endplate potentials in rat diaphragm in vitro during prolonged nerve stimulation (Wouters and Van den Bercken,

in preparation). Thus there is some doubt whether ACTH$_{4-10}$ has a direct effect on nicotinic cholinergic transmission in in vitro preparations of non-hypophysectomized animals.

In contrast to ACTH$_{4-10}$, DG-LVP was found to have no effect on slow synaptic inhibition. This is in keeping with the hypothesis that these neuropeptides have basically different actions, although their effects on avoidance behaviour are qualitatively similar (De Wied, 1977).

Answering the question of whether effects of ACTH$_{4-10}$ on slow synaptic inhibition similar to those reported here play a role in the behavioural action of ACTH$_{4-10}$ and related neuropeptides is beyond the scope of the present study. The results clearly demonstrate that ACTH$_{4-10}$ is capable of modulating synaptic transmission, probably by acting upon the postsynaptic membrane. It is conceivable that ACTH$_{4-10}$ also affects central synaptic transmission in a similar way. Since the present results show that ACTH$_{4-10}$ does not affect nicotinic cholinergic synaptic transmission, it might be concluded that the action of ACTH$_{4-10}$ is confined to catecholaminergic transmission. Such a conclusion would however be premature. More information is required to answer this question.

Acknowledgements

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