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ELECTROPHYSIOLOGICAL EFFECTS OF PEPTIDES DERIVED FROM PRO-OPIOMELANOCORTIN

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ABBREVIATIONS

ACTH	Adrenocorticotrophic Hormone	HO-DI	Homozygous diabetes insipidus
CNS	Central Nervous System	HO-NO	Homozygous normal
DALA	D-Ala ² -Leucine ⁵ -enkephalin amide	sIPSPs	Slow Inhibitory Postsynaptic Potentials
DADL	D-Ala ² -D-Leucine ⁵ -enkephalin amide	IU	International Unit
D.C.	Direct Current	LE	Leucine ⁵ -Enkephalin
DH	Dorsal Horn	LC	Late Component
DR	Dorsal Root	LS	Lateral Septum
DRPs	Dorsal Root Potentials	β -LPH	β -Lipotrophic Hormone
DT α E	Des-Tyr ¹ - α -Endorphin	LSC	Lateral Septal Complex
DT γ E	Des-Tyr ¹ - γ -Endorphin	ME	Methionine ⁵ -Enkephalin
β E	β -Endorphin	MEA	Methionine ⁵ -Enkephalin Amide
ECOG	Electrocorticogram	MR	Membrane Resistance
EEG	Electroencephalogram	MUA	Multiple Unit Activity
EPs	Evoked Potentials	N	Negative
EPSPs	Excitatory Postsynaptic Potentials	OX	Oxytocin
sEPSPs	Slow Excitatory Postsynaptic Potentials	P	Positive
FK (33-824)	(D-Ala ² , N-ME Phe ⁴ , Met-(O) ⁵ -al)-enkephalin	RMP	Resting Membrane Potential
FW (34-569)	(Me-Tyr ¹ , D-Ala ² , N-MePhe ⁴ , Met-(O) ⁵ -al)-enkephalin	SG	Sympathetic Ganglion
GABA	γ -aminobutyric acid	SWS	Slow-Wave Sleep
HE-DI	Heterozygous diabetes insipidus	TTX	Tetrodotoxin
		VPL	Ventral Posterior Lateral

1. INTRODUCTION

The pituitary gland elaborates and releases peptides and these peptides may alter various brain functions by acting within the brain. This appeared for the first time in experiments with animals whose pituitary gland had been removed. Hypophysectomized rats had an impaired acquisition and maintenance of the shuttle-box avoidance response and the impaired avoidance behavior could be corrected temporarily by injecting the animals with the adrenocorticotrophic (ACTH) or melanocyte-stimulating (MSH) hormones from the pituitary (de Wied, 1969). Subsequently it was found that fragments of these hormones, that lack the classical endocrine effects (as for example ACTH₄₋₁₀) act on behavior in intact rats, therefore these pituitary peptides must have acted at least partly within the brain. These and other experiments (de Wied, 1974) led to the hypothesis (de Wied, 1969) that pituitary hormones function as precursors for shorter peptides designated as neuropeptides and that in turn these neuropeptides act upon the brain and thus may influence its function.

In 1975 Hughes and his co-workers (Hughes, 1975; Hughes *et al.*, 1975) discovered two endogenous pentapeptides with opiate-like analgesic activity. The structure of the pentapeptides appeared to be identical with the 61-65 amino acid residues of the pituitary β -lipotropic hormone (β -LPH) molecule. These observations directed attention to β -LPH. It was soon found that the C-terminal part of the β -LPH molecule (β -LPH₆₁₋₉₁) referred to as β -endorphin (β E), resembled the enkephalins in its affinity for the stereospecific opiate receptors and in inducing analgesia (Bradbury *et al.*, 1976a, b). Subsequently, it was

suggested that β -LPH and ACTH were parts of a much larger protein (Mains *et al.*, 1977; Orth and Nickolson, 1977) histochemically detectable in cells of the pituitary gland and in some hypothalamic neurons located within the anatomical borders of the arcuate nucleus (Bloom *et al.*, 1978, 1979; Dupont *et al.*, 1980). The structure of this peptide is presented in Fig. 1 as it was first proposed by Nakanishi *et al.* (1979). In addition to β -LPH, the peptide contains the amino acid sequences of ACTH and MSH and could thus function as precursor for these peptides. The release of β -LPH, ACTH and MSH from the pituitary indeed appeared to be controlled by the same mechanism (Guillemin *et al.*, 1978). Accordingly, the peptide was named pro-opiocortin or pro-opiomelanocortin.

Exposure of β E (β -LPH₆₁₋₉₁) to enzymes associated with enriched synaptosomal plasma membranes from rat brain yielded a number of shorter peptides (Burbach *et al.*, 1980). Some of these peptides, e.g. γ -endorphin (γ E; β -LPH₆₁₋₇₇), Des-Tyr¹- γ E (DT γ E; β -LPH₆₂₋₇₇) and α -endorphin (α E; β -LPH₆₁₋₇₆) or Des-Tyr¹- α E (DT α E; β -LPH₆₂₋₇₆) could be differentiated by assessing their biological activities in various behavioral procedures (for review see de Wied and Jolles, 1982). It was therefore suggested that on its release from neurons, pro-opiocortin is degraded into fragments which modulate complex brain functions underlying behavioral adaptation (de Wied and Jolles, 1982). The alterations in brain functions that follow the administration of peptides and that can be measured with behavioral procedures should also be measurable with electrophysiological techniques. Experiments with electrophysiological techniques, and designed to examine the influence of peptides on brain processes describable in neurophysiological terms are the topic of the present review.

There are mainly historical reasons for separating the studies analysing the effects of ACTH- and MSH-related peptides from those dealing with the action of endorphins and enkephalins. Enkephalins are presumably formed from a molecule different from the pro-opiocortin molecule since enkephalin-like immunoreactivity was found in neurons that failed to react with β E antisera (Bloom *et al.*, 1978). However, the endorphins often resemble the enkephalins in their effects on single neurons, suggesting that both types of peptides may have a similar mode of action. The action of enkephalins on single cells has been analysed in great detail (for review see Frederikson, 1977; Bradley *et al.*, 1978a,b; North, 1979; Zieglgänsberger and Fry, 1978; Zieglgänsberger, 1980) and discussing the action of enkephalins may therefore be of relevance to the question of endorphins. A short discussion of the methods is included for those readers who are less familiar with procedures used in electrophysiological experiments.

2. METHODOLOGY

2.1. MACROELECTROPHYSIOLOGICAL MEASUREMENTS OF NEURONAL PROCESSES

The electroencephalogram (EEG), electrocorticogram (ECoG), evoked potentials (EPs) and multiple-unit activity (MUA) are the macroelectrophysiological measurements most often used for studying the action of drugs on the brain *in vivo*.

2.1.1. The Electroencephalogram (EEG)

This is the time history of the potential differences recorded between electrodes in contact with, or in close proximity to brain tissues, and the reference electrode placed at some remote, electrically inactive site. The EEG reflects the global synaptic activity of a large neuron population, and as such provides an index of brain function. As it is a complex parameter whose morphology changes continuously along the wakefulness-sleep continuum, the EEG allows no simple interpretation. Neuropharmacological studies investigating drug effect on this parameter are therefore mostly limited to comparisons of spontaneous EEG activity under control (no drug) conditions with the activity recorded following drug treatment. Some kind of EEG quantification is necessary for such a comparison. Until recently, EEG signals were mostly evaluated by visual inspection and manual measurement of the main variables, the amplitude and frequency. For this

FIG. 1. Primary structure of bovine pro-opiomelanocortin molecule. The molecule incorporates gamma-MSH, ACTH (corticotropin, 1-39) and beta-lipotropin (β -LPH; 42-132). The alpha-MSH (1-13) and CLIP (18-39) sequence can be recognized in the corticotropin chain. β -LPH incorporates gamma-lipotropin (42-99), the sequence of beta-MSH (82-99) and the 31 amino acid long chain of beta-endorphin (β E; 102-132). β E in turn incorporates the sequence 102-106 of enkephalin (adapted from Bloom, 1981).

Ala—Alanine
Arg—Arginine
Asn—Asparagine
Asp—Aspartate
Cys—Cysteine
Gln—Glutamine
Glu—Glutamate
Gly—Glycine
His—Histidine
Ile—Isoleucine

Leu—Leucine
Lys—Lysine
Met—Methionine
Phe—Phenylalanine
Pro—Proline
Ser—Serine
Thr—Threonine
Trp—Tryptophan
Tyr—Tyrosine
Val—Valine

purpose, the frequency scale was divided into a number of frequency bands which were given the following Greek notation: delta band, for the activity under 3.5 Hz; theta band, for activity from 3.5 to 7.0 Hz; alpha band, frequencies from 8 to 13 Hz and beta band, for activity above 13 Hz. Visual analysis consisted in determining the amount and the peak-peak amplitude of activity in these bands by measuring the peaks in the polygraph records. In addition to these quantitative expressions, a number of qualitative terms are used to describe the wave-forms in the EEG, e.g. spike and wave complex, K-complex, Mu-rhythm, spindles etc. Storm Van Leeuwen *et al.* (1966) and Chatrian *et al.* (1974) have provided glossaries of the terms used in animal and clinical electroencephalography. A number of these terms, e.g. synchronization, desynchronization, high voltage slow activity (HVSA), low voltage fast activity (LVFA), spindling are still commonly used in psychoneurophysiological and neuropharmacological descriptions of the EEG.

Digital computers have greatly improved the objectivity and resolution of EEG analysis. The speed and capacity of the computers to process several EEG channels in real time stimulated the development of new computer-assisted methods (Burch, 1959; Storm Van Leeuwen, 1964; Byford, 1965; Schallek *et al.*, 1967; Fink, 1968; Fairchild *et al.*, 1971; Matousek, 1973; Itil, 1974; Künkel, 1977; Matejcek and Devos, 1976; Haller, 1979) for quantifying the EEG. These methods consist in computing some kind of EEG estimate describing the amplitude and frequency of the EEG signals using a single set of numbers that is readily amenable to further statistical comparisons. Automatized EEG analysis has opened a new approach in CNS pharmacology; quantitative pharmacoelectroencephalography. This approach evaluates drug action according to the significance of changes that occur in various frequency bands of the spontaneous human EEG following administration of the drug to be tested. It was quantitative pharmacoelectroencephalography which led to the finding that therapeutically equivalent compounds, even if they are pharmacologically different, will still produce similar changes in the spontaneous human EEG (Itil, 1974). Thus, drugs classified as tranquillizers according to their clinical effects had EEG profiles that contained significantly lower amounts of the very low (delta, theta and alpha) frequencies and greater proportions of the fast frequencies (Schallek and Keuhn, 1959; Fink, 1968; Joy *et al.*, 1971; Itil, 1974) than did 'placebo profiles'. The neuroleptics (major tranquillizers) enhanced the production of slow (delta, theta) waves and suppressed the fast activity (Fink, 1968; Itil, 1974), whereas most of the psychostimulants (thymoleptics) decreased the slow delta activity and decreased the intensity of the EEG signal (Fink, 1974). Interestingly, the EEG profiles of diazepam, chlorpromazine and α -amphetamine found in cat EEG (Haller, 1979) were similar to those based on the human EEG, suggesting that the action of many psychoactive agents on brain may be similar across species and that the results obtained in the animal studies may be relevant to humans.

2.1.2. Evoked Potentials (EPs)

Measuring different kinds of cortical EPs before and after administering centrally acting drugs is another macroelectrophysiological approach to the study of drug action on the brain (for review see Shagass, 1977). Cortical evoked potentials, sometimes also termed the event-related potentials, are EEG segments which are time-locked to a repetitive (sensory) stimulus. Averaging of these segments is often necessary to increase the signal-noise ratio, so that the evoked potential is sufficiently enhanced to become visible against the background EEG. An EP normally consists of a number of positive and negative waves (components) that appear at different latencies following the stimulus. The drug-induced alterations in amplitude, form and latencies of these components may therefore be used for evaluating the drug effect on the brain. Saletu (1974) examined the human somato-sensory EPs following treatment with various psychoactive agents. A standard procedure was used for inducing the EPs: identical placement of the recording electrodes, controlled level of vigilance and identical data processing. The drugs used for similar clinical purposes produced similar alterations in EPs and the authors thus

suggested, that this method was applicable for classification and/or prediction of the therapeutic effects of new psychoactive agents.

Different waves in the EPs are presumably related to different neuropsychological and/or psychophysiological processes. Several early (with latency shorter than 60 msec) and late (latency longer than 60 msec) waves could be distinguished in EPs that could be recorded above the somatosensory cortex following stimuli applied to the medial nerve. According to Allison *et al.* (1977) the positive wave P15 that occurs in EPs at 15 msec after a stimulus is related to the volley in afferent fibers terminating on neurons of the ventrobasal posterior lateral (VPL) thalamic nucleus, to the synaptic events in this nucleus and to the action potentials in the thalamo-cortical fibers. The subsequent negative-positive waves with latency between 20–60 msec reflect the primary response in the somato-sensory cortex. An effect of drugs on these short-latency components would therefore indicate an alteration in synaptic transmission in the thalamus or in the primary somato-sensory areas. The late components, e.g. the positive P180 wave in these EPs could be markedly augmented by asking the subjects to concentrate fully on the stimulus (Satterfield, 1968), suggesting that the magnitude of these components expresses the amount of selective attention in these subjects. The drug-induced alteration in P180 and other late components could thus be interpreted as a drug effect on selective attention.

2.1.3. Multiple-Unit Activity (MUA)

For technical details of this technique see, e.g. Buchwald *et al.*, 1973. This is a measure of the frequency of action potentials that could be recorded extracellularly and within the selected time interval from an unknown population of spontaneously active or stimulus-driven neurons. Like the EEG, the frequency of MUA varies with fluctuations in the subject's vigilance and thus may be a useful means for signalling the action of drugs on the vigilance regulating systems. It should be realized that the stimulus- or the drug-induced excitation of one kind of neurons in one structure is likely to be associated with inhibition of other kinds of neurons within the same structure. MUA will register only a net change in total number of action potentials per unit time; this change could be nil, close to zero or so small that it remains buried in the fluctuations of 'baseline' activity. It is perhaps for this reason that MUA is not frequently used in neuropharmacological studies.

2.2. MICROELECTROPHYSIOLOGICAL MEASUREMENTS OF NEURONAL PROCESSES

Microelectrophysiological techniques allow the action of compounds to be studied at the subcellular level, either *in vivo* or *in vitro*. Studies *in vivo* usually involve extracellular recordings of action potentials from the spontaneously or stimulus-driven single neuron before, during and after administration of a test compound. The compound may be given systemically (s.c., i.p. or i.v.), injected in small volumes into the intracerebroventricular system, administered directly into a selected brain structure, or applied by micro-iontophoretic or pressure injections in the vicinity of the neurons being used for recording. Compounds that pass through the blood-brain barrier can be given systemically. This route of administration may be preferred for its simplicity and because the compound can be given in doses which induced the desired behavioral effects. While i.c.v. injections circumvent the blood-brain barrier, one cannot decide whether the compound has changed neuronal activity by acting directly on the recorded neuron or at some other place remote to the recording site. An intracerebrally administered substance acts primarily at the locus of injection. It should be kept in mind that the effects following administration via this route may be merely pharmacological or toxic effects of the drug and unrelated to the behavior in question. The microiontophoretic procedure (for details see Curtis, 1964) consists of expelling drug ions from one barrel of a micropipette filled with an aqueous solution of the drug by passing through this barrel a small current of the same polarity as the ions, and concomitantly recording the activity of the neuron through another barrel of the micropipette.

In vivo iontophoretic drug application is superior to any other route of administration in that it eliminates the interference of the blood-brain and enzymatic barriers and allows the neurons to be studied under almost natural conditions with intact synaptic inputs. The value of microiontophoresis increases further if the cells under study can be identified functionally (by specific stimuli) or electrophysiologically (using electrical stimulation of known synaptic inputs). However, it is very difficult to determine and to control the actual concentration of iontophoretically applied drugs at the receptor sites. This is due to many factors (for review see Bloom, 1975) some of which, e.g. diffusion, metabolic degradation and uptake of the drug by neural tissue, cannot be controlled by the investigator. Microiontophoresis is therefore less suited for pharmacological studies involving dose-response relationships, agonist-antagonist interaction or for comparing relative potencies of drugs.

2.2.1. Studies In Vitro

Neural tissues maintained *in vitro* provide an excellent preparation for use in pharmacological experiments as the drug concentration in the incubating medium also becomes the actual concentration of the substance at the receptor sites. In addition, the *in vitro* arrangement permits long-term intracellular recordings from single neurons without interference by anesthesia or transsynaptic activities and allows the ionic mechanisms of drug action to be elucidated. Results obtained *in vitro* must eventually be confirmed *in vivo* as neurons *in vitro* may behave differently from those *in situ*.

3. EFFECTS OF ACTH/MSH PEPTIDES ON MACROELECTROPHYSIOLOGICAL MEASUREMENTS

3.1. EEG

The EEG was first used to study the central action of peptides in experiments on the influence of ACTH on experimentally induced seizure-like activity. A single injection of ACTH (1–8 mg/kg) to rats could significantly lower the threshold for induction of epileptoid seizures and convulsions produced with pentamethylene tetrazol (Torda and Wolff, 1952a). Adrenalectomy or hypophysectomy had no effect on the response to ACTH indicating a direct, extra-adrenal effect of ACTH in the brain, Wasserman *et al.* (1965) elicited seizure activity with an electric shock delivered transcorneally to intact or adrenalectomized rats maintained on a sodium rich diet. Less current was needed to elicit seizures in both experimental groups if they had received 5 IU of ACTH daily for one week prior to the test. ACTH also increased the intracellular Na^+ concentration in brain, regardless of whether or not the adrenals had been removed. Thus, ACTH may have affected the susceptibility to develop seizures by interfering with processes which regulate the cellular distribution of Na^+ . Donaldson *et al.* (1971) found that the intracerebral administration of ouabain, a Na^+ pump inhibitor, induced seizure activity in rats. Interestingly, the onset of the ouabain-provoked seizures could be greatly accelerated by prior treatment of the rats with α -MSH (Izumi *et al.*, 1973). In contrast to experimentally induced epilepsy in animals, which was enhanced by ACTH (see also Woodbury, 1958), human epilepsy was only aggravated by ACTH in patients suffering from the grand mal form of the illness. In fact, treatment of young (under 15 years of age) patients with ACTH ameliorated the clinical and electrophysiological symptoms of petit mal epilepsy in nearly 65% of the cases (Gestaut *et al.*, 1959). According to EEG records obtained from 143 petit mal epileptic children, 23% of the patients were cured following a 10 day treatment with ACTH, 43% exhibited marked improvement and the symptoms showed no change or worsened in 34% (Klein, 1970). Different mechanisms for experimentally induced seizure activity and pathological human epilepsy (Schmidt and Wilder, 1968) may perhaps account for the dissimilar action of ACTH on this abnormal activity in experimental animals and in humans. Nevertheless, these and other studies (Glaser, 1953; Glaser *et al.*, 1955) have shown that ACTH may change brain electrical activity in both humans and experimental animals by acting directly upon the brain.

Physiological EEG activity could also be altered by peptides related to ACTH. Kawakami *et al.* (1966) recorded the spontaneous EEG from several subcortical structures in freely moving rabbits. In many structures the frequency composition of the spontaneous activity changed after i.v. injections of ACTH. Thus, treatment with the peptide decreased the theta and 20–60 Hz activities in the hippocampus and increased the amounts of 4–13 and 20–60 Hz activities in the baso-lateral amygdala. The anterior hypothalamic area was dominated by the slow 4–13 Hz activity. The initial increase in the 2–8 Hz band which occurred in the medial preoptic area in the first 60 min after the injection was later replaced by a marked decrease in this band. The activities in the median eminence and arcuate nucleus remained depressed for 3 hr after ACTH was given. ACTH did not change the EEG of the posterior hypothalamic area and mamillary bodies but slightly increased the 4–8 Hz waves in the mesencephalic reticular formation. The frog preoptic area generated more 4–9 c/sec waves after α -MSH injection to the dorsal lymph sac than prior to the treatment (Denman *et al.*, 1972). However, Sawyer *et al.* (1968) saw only neocortical desynchronization subsequent to 2 IU of ACTH, and Pfaff *et al.* (1971) failed to detect any alterations in cortical EEG records even after injecting the rats with 10–20 IU of the peptide. On visual inspection of EEG records, Dyster-Aas and Krakau (1965) found the marked increase in slow wave cortical activity subsequent to s.c. injection of 10 mg α -MSH to intact rabbits. The natural α -MSH and β -MSH, as well as the synthetic α -MSH, promoted the 4–9 Hz slow activity in the parietal EEG of rats (Sandman *et al.*, 1971). In a double blind experiment on six healthy volunteers, panhypopituitarism patients and two patients with Sheeman's syndrome, the i.v. infusion of saline containing 10 mg of α -MSH slowed down the spontaneous occipital EEG (Velasco *et al.*, 1972). Recently, Miller *et al.* (1981) re-examined the action of α -MSH on the spontaneous EEG of rats and used a more objective spectral analysis for quantification. The occipital power spectra of rats treated i.p. with 80 μ g/kg of the peptide had a markedly increased intensity in the 2–5 Hz band and a mildly increased power in the 7–10 Hz band. In the EEG recorded sagittally at Cz and Oz positions in the scalp of elderly male and female volunteers suffering from mild symptoms of organic brain disease, 30 mg of ACTH_{4–10} given s.c. significantly increased the proportion of delta (1–3 Hz) and theta (3–7 Hz) activities and decreased the amount of α and β activities. The female patients in this study appeared to be significantly more sensitive to ACTH_{4–10} than the male subjects (Braconnier *et al.*, 1979).

Diversity in the experimental procedures must account at least in part for the great diversity in EEG patterns obtained in these experiments with peptides. Rockstroh *et al.* (1981) examined the effect of the orally active ACTH_{4–9} analog (Org 2766) on the frontal, precentral and parietal EEG recorded from young healthy volunteers during 6 sec epochs separating the warning and the test stimuli. In this case, the ACTH analog significantly increased the low (8–10 Hz) alpha frequencies and decreased the intensity of the signal in the 2–24 Hz band. In a different test situation (Miller *et al.*, 1974), ACTH_{4–10} significantly decreased the activity in the 3–7 Hz band but augmented the duration and amplitude of activity in the 7–12 Hz band and in the band above 12 Hz. Clearly, in many cases, the peptides themselves did not elicit a particular EEG pattern just as they did not elicit a particular behavioral pattern (for review see de Wied and Jolles, 1982). They only modified the behavioral and EEG patterns induced by a particular experimental procedure.

Repeated presentation of a familiar tone to human volunteers elicited α -synchronized activity in the occipital scale EEG. ACTH_{1–24} and ACTH_{1–10} i.v. in doses of 1–2 mg could suppress this stimulus-induced α -synchrony. The ACTH_{11–24} sequence, which was only weakly active in behavioral procedures (de Wied, 1979), failed to affect this measure (Endröczy *et al.*, 1970). In another double blind study on 20 healthy young volunteers, ACTH_{4–10} mg injected i.v. was also effective in blocking the stimulus-induced occipital alpha rhythm in the peptide-treated subjects (Miller *et al.*, 1974). Thus, the peptides that elicit similar effects in the same behavioral test situation may elicit similar effects in the same electrophysiological test.

ACTH and related peptides delay the extinction of avoidance behavior, presumably by acting on the midbrain limbic system (for review see de Wied and Jolles, 1982).

Hippocampal theta activity is an excellent macroelectrophysiological parameter for studying the functioning of this system. This rhythmic field is generated by a synchronous input arriving at the hippocampal formation from the septum (Petsche *et al.*, 1962; Gogolak *et al.*, 1967, 1968). In order to produce a synchronous input, the septal network needs excitation from the brain stem afferents (Green and Arduini, 1953; Klemm, 1970, 1971, 1972a, b; Paiva *et al.*, 1976). Urban and de Wied (1976) elicited theta rhythm in the hippocampus and posterior thalamus of freely behaving rats by stimulating the mesencephalic reticular formation with constant stimuli at 100 Hz. Treating the rats with ACTH₄₋₁₀ 150 µg/kg body weight increased the peak and mean frequencies of the stimulus-induced hippocampal synchrony by approximately 0.5 Hz (see Fig. 2). The peptide-induced acceleration of theta rhythm appeared 30–60 min following the injection and persisted for several hours. A similar acceleration of hippocampal rhythmicity was obtained by mildly (10 µA) increasing stimulus intensity. On the assumption that the stronger stimulus engaged a greater number of mesencephalic units in generating theta rhythm it was suggested that ACTH₄₋₁₀ accelerated hippocampal activity by increasing the excitability of those units.

Theta activity occurs spontaneously in moving rats or during paradoxical sleep (PS). In moving rats, the frequency of this activity may vary rapidly between 6–12 Hz depending on the velocity and intensity of the movement (Whishaw and Vanderwolf, 1973). The frequency of the theta rhythm which accompanies PS is similar to the electrically elicited rhythmicity in that it is very stable, remaining unchanged for most of the duration of PS episodes. The large narrow peak at 7.5–8.0 Hz and the negligible amounts of activity in other frequency bands in the power spectra of PS activity (Urban, 1981) indicate uninterrupted, intense and presumably exclusive activation of the midbrain–septo-hippocampal system during the PS episodes. These episodes are therefore very well suited for examining the effect of ACTH₄₋₁₀ on the midbrain limbic system under physiological conditions. Urban and de Wied (1975) found that during PS the mean theta frequency in Brattleboro rats homozygous for hypothalamic diabetes insipidus (HO-DI) was 0.7–1.0 Hz lower than that of the heterozygous litter mates (HE-DI) or homozygous normal (HO-NO) rats. Microinjections i.c.v. of 20 ng ACTH₄₋₁₀ temporarily raised the rate of hippocampal rhythmicity of HO-DI rats to the levels found in HE-DI or HO-DO rats. The same dose of ACTH₄₋₁₀ administered to HE-DI rats increased further the amounts of high theta frequencies in these animals (Urban and de Wied, 1978). Thus, irrespective of the dose and route of administration, ACTH₄₋₁₀ accelerated both electrically elicited and PS-induced theta activities.

There has been no detailed investigation of the relationship between the structure of peptides and their capacity to accelerate hippocampal rhythmicity. Two important pieces of evidence however suggest that the relationship may resemble that revealed in avoidance behavior procedures. The amino acid residue phenylalanine in position 7 is essential for the effect of ACTH₄₋₁₀ on extinction of avoidance behavior (de Wied, 1969). When the phenylalanine residue was replaced by its D-enantiomer the peptide (D-Phe⁷) ACTH₄₋₁₀ was generated. It had effects on avoidance behavior opposite to those of the non-substituted, all L ACTH₄₋₁₀ and facilitated extinction of the pole jumping response. When injected in the same amount as ACTH₄₋₁₀, (D-Phe⁷) ACTH₄₋₁₀ slowed down PS hippocampal theta rhythm. ACTH₁₁₋₂₄ was only weakly active in avoidance procedures (de Wied, 1979). ACTH₁₁₋₂₄, 20 µg injected i.c.v. failed to alter the frequency composition of hippocampal activity during PS (Urban and de Wied, 1978).

The frequency composition of the spontaneous and slow wave sleep (SWS) hippocampal EEGs is much more complex than that of the stimulus or the PS-induced theta synchronies. In addition to theta activity, they contain a large quantity of delta (1–4) Hz waves and significant amounts of frequencies above 12 Hz. Several other systems in addition to the theta producing network must therefore participate in the genesis of these EEGs. Interestingly, the spontaneous EEGs recorded from the rat hippocampus during the intervals separating the stimulation trials (Urban and de Wied, 1976) or during SWS episodes (unpublished observation) were little affected by ACTH₄₋₁₀. A striking feature of

FIG. 2. Effect of subcutaneously injected ACTH₄₋₁₀ (150 $\mu g/kg$) on the mean power spectrum ($\pm$ S.E.M.) of the dorsal hippocampus and posterior thalamus theta activity induced by stimulating (50 μA) the mesencephalic reticular formation of a freely behaving rat. Inset in A depicts the localization of the recording electrode in the dorsal hippocampus (HID); upper inset in B illustrates the placement of the recording electrode in the posterior thalamus, lower inset in B indicates placement of the stimulating electrodes (according to Urban and de Wied, 1976).

these EEGs was that the amounts of activity in various frequencies fluctuated markedly from episode to episode and that the magnitude of the fluctuations in one particular frequency band could largely exceed the magnitude of any change that ACTH₄₋₁₀ could induce in the theta band. Any effect of the peptide under these conditions would presumably have stayed below the detection limit. Two important conclusions follow from these experiments. First, the effects of ACTH-like peptides on a particular brain structure or system should be studied with the structure or system in full functional activity. Second, the action of these peptides is best studied with an experimental procedure in which only one structure or system is selectively activated at any one time.

ACTH₄₋₁₀ given s.c. significantly slowed down hippocampal theta rhythm, in a dog put in an operant condition test situation (Urban *et al.*, 1974). Studies on rats suggested that the action of ACTH₄₋₁₀ on the theta rhythm-producing midbrain limbic system is most probably an excitatory one. If the peptide were acting solely on this system, hippocampal theta frequency would be expected to increase following the injection of ACTH₄₋₁₀. The opposite was found. Thus, in slowing down hippocampal rhythmicity, the peptide must have acted on, and promoted activity in structures inhibitory on the midbrain limbic system. The raphe nucleus might be one of these structures. Electrical stimulation of the raphe nucleus suppressed or completely abolished hippocampal synchronized activity (Maru *et al.*, 1979; Yamamoto *et al.*, 1979).

The qualitatively different effect of ACTH₄₋₁₀ on hippocampal theta activity of rats and of dogs is perhaps another example of the capacity of the peptide to alter the functioning of brain systems or structures that were activated by a particular behavioral procedure. When the animal is awake, a sudden increase in the frequency of hippocampal theta activity is always associated with behavioral disinhibition, i.e. readiness of the animal to respond by movement to biologically significant stimuli. The decrease in the rate of hippocampal synchrony reflects behavioral inhibition and diminished readiness of the animal to move (Kamp *et al.*, 1971; Wishaw and Vanderwolf, 1973; Black, 1976; Arnolds *et al.*, 1979a,b). ACTH₄₋₁₀ slowed down theta rhythm in a dog during behavioral episodes described as 'facing', i.e. attentively awaiting the onset of the conditioned stimulus announcing food presentation. In a mildly food-deprived animal, movement inhibition was thus the behavior relevant to the situation. It is therefore conceivable that the systems that elicit behavioral inhibition, and not the structures initiating movement must have been active for most of the duration of 'facing' episodes. The fact that ACTH₄₋₁₀ induced slowing down of hippocampal theta activity during the 'facing' episodes indicates that the peptide increased the functional efficiency of the movement-inhibiting system and not that of the movement-initiating system.

A different action of ACTH-like peptides on the hippocampus was shown by Valero *et al.* (1977). They administered stimuli of different frequencies to the septum and measured the intensity of the current needed to elicit theta-like EEG synchrony. In untreated male rats, the 7.7 Hz stimulus required the lowest current for inducing hippocampal synchrony. ACTH and ACTH₄₋₁₀ shifted the minimal current to 6.9 Hz.

3.2. EVOKED POTENTIALS

ACTH/MSH peptides may alter the EPs in various brain structures in experimental animals and human subjects. In one study on chickens, Koranyi and Endröczy (1970) found that ACTH and ACTH₄₋₁₀ attenuated by approximately 50% the cortical EPs elicited in these animals by stimuli delivered to the midbrain reticular formation. In rats, light flashes induced EPs in the occipital cortex. When the light-induced EPs were averaged, there appeared several distinct components in these potentials. ACTH₄₋₁₀ (50 µg/kg) had little effect upon the early components of the EPs but the peptide significantly attenuated the late positive-negative (P3, N3) waves in EPs irrespective of the stimulus intensity used to elicit these potentials. The (D-Phe⁷) ACTH₄₋₁₀ analog had a similar but weaker action on the late P3 and N3 waves (Wolthuis and de Wied, 1976). According to some investigators (Velasco *et al.*, 1968), the decrease in amplitude of the late components (LC) in EPs is associated with an increase in the discharge rate of the reticulo-thalamic neurons whereas the increase in magnitude of LC reflects the decrease of neuronal activity within the system. Thus, ACTH-like peptides may have attenuated the LC in the cortical EPs by acting upon the non-specific reticulo-thalamic ascending systems and by increasing excitability of neurons belonging to this system.

Kastin *et al.* (1971) and Velasco *et al.* (1972) elicited complex EPs above the somatosensory cortex by stimulating the median nerve in fully attentive subjects (patients were instructed to respond by pressing a lever as soon as possible), during episodes of inattentiveness (the subjects were asked to remain quiet and ignore the stimulus) and in patients whose attention was drawn elsewhere (the stimulus was presented while the

subjects were solving a verbal memory and visual retention test), i.e. 'distracted' subjects. EPs obtained from fully attentive subjects were characterized by the presence of late P4 and P5 waves of considerable amplitude. The inattentiveness of patients markedly diminished the size of these components and the distraction almost completely abolished the P4 and P5 components in the somatosensory EPs. A slow i.v. infusion of α -MSH failed to alter the procedure-dependent incidence of P4 and P5 waves. However it diminished the latency and markedly augmented the size of these waves in EPs. The peptide-induced increase in magnitude of P4 and P5 was appreciably greater in the attentive subjects than in the inattentive ones, suggesting some influence of the peptides on attention processes. Rockstroh *et al.* (1981) studied the effect of the ACTH₄₋₉ analog Org 2766 on human scalp acoustic EPs in a situation that required full attention from the subjects. The EPs elicited by acoustic stimuli above the frontal and parietal cortices showed an early negative N100 and late positive P300 waves. ACTH₄₋₉ (Org 2766), 40 mg orally, decreased the latency and increased the magnitude of both N100 and P300. Thus, in two similar situations, both ACTH₄₋₁₀ and ACTH₄₋₉ affected two kinds of scalp EPs in a similar fashion i.e. decreased the latency and increased the amplitude of the principal late components.

When the task was more complex however, the effect of peptides on late components in EPs was much altered. Miller *et al.* (1976) presented irrelevant and relevant visual stimuli to their subjects. The subjects were asked to press a button only on appearance of the relevant stimulus. EPs above the occipital cortices were recorded with both kinds of stimuli. Irrespective of the relevance of the stimulus, ACTH₄₋₁₀ (30 mg s.c.) increased the latency and decreased the amplitude of the late negative and positive components which were visible in EPs at latencies of about 250 and 400 msec. In addition, after treatment with the peptide, a new small negative component of EPs appeared about 350 msec following the stimulus. Fehm-Wolfsdorf *et al.* (1981) studied the effect of ACTH₄₋₉ when two other types of stimulus situations were used. They presented one of two acoustic S1 stimuli (400 or 1200 Hz tone) for 6 sec. Each S1 stimulus was followed by a neutral (600 Hz) or an aversive (110dB noise) S2 stimulus. The subjects were instructed to terminate the S2 stimuli by operating a microswitch. ACTH₄₋₉ (Org 2766) failed to alter the latency or amplitude of the late N100 and other waves in EPs elicited above the frontal and parietal cortices by the S1 stimulus. The P300 component which was augmented in the one-stimulus situation (Rockstroh *et al.*, 1981), had a diminished amplitude in the two-way stimulus situation.

It has been hypothesized that ACTH/MSH-like peptides enhance attentional processes selectively (Kastin *et al.*, 1971). One argument in favor of such an interpretation was the fact that the P4 and P5 waves in the somatosensory EPs whose amplitude was augmented by peptides, were most pronounced in the fully attentive subjects and absent in the 'distracted' individuals (Kastin *et al.*, 1971; Velasco *et al.*, 1972). Indeed, in a discriminative test situation designed to study selective attention and which resembled in many features that used by Fehm-Wolfsdorf *et al.* (1981), the P300 wave was much enhanced when the subjects were attentive (Hillyard *et al.*, 1973). However, the peptide-induced enhancement of P4 and P5 waves in the somatosensory EPs (Kastin *et al.*, 1971) and of N100 and P300 waves in the frontal and parietal acoustic EPs (Rockstroh *et al.*, 1981) was found only in a simple stimulus-response situation. In the two-stimulus test situation which required perhaps even a higher degree of attention and vigilance, ACTH₄₋₁₀ increased the latency and decreased the amplitude of the principal (negative and positive) late components (Miller *et al.*, 1976), and ACTH₄₋₉ suppressed the P300 wave (Fehm-Wolfsdorf, 1981). There is increasing evidence that the late components in the cortical EPs reflect, in addition to selective attention many other physiological processes (for review see John and Schwartz, 1978; Näätänen, 1975). Any attempt to interpret the peptide-elicited changes in morphology of late components as an action of the peptide on selective attention is therefore certainly a great oversimplification.

3.3. MULTIPLE-UNIT ACTIVITY

Four studies were found in which MUA was used for investigating the influence of

ACTH and related peptides on the brain. ACTH (2 IU) injected s.c. in rats elicited a slight increase in MUA recorded through indwelling metal semi-microelectrodes in the zona incerta and arcuate nucleus. Injections of $10\ \mu\text{g}$ ACTH₁₋₂₄ into the cerebroventricular system of rabbits elevated the frequency of the MUA in the lateral preoptic and diagonal band of Broca areas. ACTH₄₋₁₀ failed to affect activity in this region (Baldwin *et al.*, 1974). In cats, ACTH increased MUA only in the mesencephalic reticular formation, but limbic forebrain activities measured in the septum in the medial preoptic area were slightly decreased by this peptide (Koranyi *et al.*, 1971a, b).

4. EFFECTS OF ACTH/MSH PEPTIDES ON MICROELECTROPHYSIOLOGICAL MEASUREMENTS

4.1. SINGLE NEURONS *IN VIVO*

ACTH and some related peptides may enhance the spontaneous or the stimulus-induced activity of single neurons in several neurons in several brain structures. Thus, the firing rate of hypothalamic neurons increased shortly (2–3 min) after i.v. injection of ACTH (Van Delft and Kitay, 1972) just as did the activity of some hippocampal cells that could be recorded before and after the i.p. injection of 10–50 IU of ACTH (Pfaff *et al.*, 1971). Microiontophoretic administration of ACTH yielded results similar to those of systemic injections. Steiner (1970) observed increased activity in a small number of diencephalic neurons during the iontophoretic release of ACTH in the vicinity of these neurons. In the medulla also, the spontaneous activity of some otherwise unidentified neurons was seen to increase in a dose-dependent manner during iontophoresis of ACTH₁₋₂₄ onto these neurons (Gent and Normanton, 1980). In this experiment, the shorter amino acid sequences such as ACTH₁₋₁₀, ACTH₄₋₁₀ or ACTH₄₋₇ did not change the firing rates of the neurons even when the peptides were applied with currents of up to 200 nA for several minutes. The rate of firing of the hippocampal neurons did not change during direct, iontophoretic administration of the whole ACTH sequence. When the peptide was applied simultaneously with noradrenaline (NA), it antagonized the inhibitory action of NA on these cells (Segal, 1976). The ineffectiveness of ACTH₄₋₁₀ on neuronal activity is surprising since this peptide accelerated the rate of hippocampal theta rhythm in rats (Urban and de Wied, 1976), diminished the amplitude of the cortical EPs in these animals (Wolhuis and de Wied, 1976) and affected a number of other macroelectrophysiological measurements in animals and humans (see Sections 3.1 and 3.2 of this review; also Sandman and Kastin, 1981). Iontophoretic structure–activity experiments on neurons in other brain regions are needed in order to identify the site(s) within the ACTH molecule which is (are) responsible for the excitatory properties of ACTH within the brain.

Systemically injected β -MSH increased the number of intracellular measured action potentials elicited in α -motoneurons in the spinal cord of cats by paired stimuli administered to the dorsal roots. This effect was visible within 2–3 min after the injection and persisted during the rest of the experiment (Krivoy and Zimmerman, 1977). The resting membrane potential, synaptic delay, magnitude and rise time of the excitatory postsynaptic potentials were not altered by the peptide. Unfortunately, other parameters of membrane properties were not examined in this experiment and thus the mechanism and locus of this action of β -MSH on α -motoneurons still remain unclear.

4.2. EXPERIMENTS *IN VITRO*

Henkin *et al.* (1973) measured the Na^+ and K^+ currents in squid giant axons before and after exposure of the axons to 80 IU ACTH. The peptide did not change the ionic current–membrane potential relationship suggesting that ACTH has little or no effect on the basic ionic events that give rise to action potentials. ACTH and ACTH₄₋₁₀ also did not affect the resting membrane potential (RMP) of rat diaphragm muscle fibers *in vitro*. This parameter remained unchanged following superfusion of the fibers with 10^{-7} M concentrations of the peptide (Birnberger *et al.*, 1977). The sucrose gap technique (for details see Nishi and Koketsu, 1968; Koketsu, 1969; Wallis *et al.*, 1975) permits quasi-intracellular

recording from a population of neurons. A shift in the D.C. potential measured across the gap indicates alterations in the RMP of these cells. RMP recorded in this way from frog sympathetic ganglion (SG) neurons showed no appreciable treatment-related fluctuations following perfusion of ganglia for 60 min with 10^{-6} M ACTH₄₋₁₀ (Wouters and Van den Bercken, 1979a). An action of peptides on RMP of the brain cells maintained *in vitro* has not been reported.

Early *in vivo* experiments have suggested that there were effects of ACTH on neuromuscular transmission (Torda and Wolf, 1952b; Strand *et al.*, 1973/4; Strand and Cayer, 1975; Strand and Smith, 1980). This action has recently been reinvestigated with the isolated phrenic nerve–diaphragm muscle preparation. In neuromuscular preparations, electrical stimulation of the afferent nerve fibers releases acetylcholine from the terminals of these fibers. The acetylcholine interacts with receptors on the adjacent postsynaptic muscular membrane and elicits end-plate potentials (EPPs). The magnitude of the EPPs is related to, amongst others, the amount of acetylcholine released from the nerve terminal. Birnberger *et al.* (1977) saw the amplitude of EPPs decrease after they added 10^{-7} M ACTH to the perfusion medium. The same or a stronger (10^{-6} M) concentration of ACTH₄₋₁₀ was without effect on EPPs (Birnberger *et al.*, 1977; Wouters *et al.*, 1979c). In the absence of changes in RMP, the peptide-induced attenuation of the EPPs could have been due to presynaptic suppression by ACTH of acetylcholine release or to interference of the peptide with postsynaptic processes at the receptor site. ACTH₄₋₁₀ increased the frequency of miniature end-plate potentials (MEPPs) in diaphragm from normal rats and in gastrocnemius muscle fibers isolated from hypophysectomized rats (Birnberger *et al.*, 1977; Strand and Smith, 1980). The MEPPs are small, randomly occurring fluctuations in the end-plate potential of unstimulated muscle fibers. They are produced by quanta of acetylcholine spontaneously liberated from presynaptic terminals (Katz, 1966). A change in frequency of these potentials therefore always indicates a presynaptic mechanism. Differences in the size of MEPPs could be due to a different size of transmitter quanta, thus having a presynaptic origin. However, the magnitude of MEPPs may also change as a result of postsynaptic processes altering the transmitter–receptor interaction. The magnitude of MEPPs was not affected by peptides (Birnberger *et al.*, 1977; Wouters *et al.*, 1979c). Thus, when diminishing the size of EPPs and augmenting the frequency of MEPPs, ACTH and ACTH₄₋₁₀ were presumably acting on presynaptic terminals.

On electrical stimulation of the afferent fibers fast excitatory postsynaptic potentials (EPSPs) comparable to the muscular EPPs are elicited in neurons of the frog sympathetic ganglion (SG). The fast EPSPs may be followed by slow excitatory postsynaptic potentials (sEPSPs) or by slow inhibitory postsynaptic potentials (sIPSPs) depending upon the type of afferents stimulated (for details see Libet *et al.*, 1968; Weight and Padjen, 1973; Weight, 1974; Nishi, 1976). The fast and slow excitatory postsynaptic potentials are mediated by the same transmitter, acetylcholine. The interaction of acetylcholine with the nicotinic cholinergic synapses elicits the fast EPSPs whereas induction of the sEPSPs requires activation of the atropine-sensitive synapses. It could be inferred from the unaltered magnitude of the fast EPSPs that ACTH₄₋₁₀ had little effect on the cholinergic transmission mediated by the nicotinic synapse. However, the peptide affected processes at the muscarinic cholinergic synapse. Functioning of the muscarinic synapse is best studied in a ganglion in which transmission through the nicotinic synapse is suppressed with nicotine or D-tubocurarine. In the bullfrog SG superfused with Ringer solution containing one of these blocking agents, a short application of the cholinergic receptor agonist metacholine induced in the SG cells a response consisting of a hyperpolarization–depolarization sequence. Perfusion of the nicotinized ganglia for 60 min with 10^{-7} M ACTH₄₋₁₀ in Ringer solution attenuated the hyperpolarization and markedly augmented the depolarization in the metacholine-induced response (see Fig. 3). The alterations in response were usually noticeable 30–60 min after the start of peptide administration and remained for the rest of the experiment. This presumably purely postsynaptic effect of ACTH₄₋₁₀ could only be partly confirmed by Wouters and Van den Bercken (1979a). They saw a tendency of the acetylcholine-induced response to increase in presence of ACTH₄₋₁₀ in some of the ganglia

FIG. 3. Effect of 10^{-7} M ACTH_{4-10} on metacholine response in curarized (50 mg/1 D-tubocurarine) bullfrog sympathetic ganglia.

only. However, the control depolarizations in their preparations were very small and difficult to measure accurately.

The size of sIPSPs elicited in SG by electrical stimuli was markedly enhanced by ACTH_{4-10} , 10^{-8} M (Wouters and van den Bercken, 1979a). There is evidence (Libet and Tosaka, 1970) implicating dopamine in the genesis of the sIPSPs. Indeed, a short application of this transmitter elicited a transient hyperpolarization in SG neurons (McAfee and Greengard, 1971). The dopamine-induced hyperpolarization significantly increased in SG exposed to ACTH_{4-10} (Wouters and Van den Bercken, 1979a). The ACTH_{4-10} effects on synaptically and on chemically elicited postsynaptic activity followed a very similar time course. This may indicate that the two types of effects have a similar mechanism.

Thus, *in vitro*, ACTH_{4-10} acted on both post- and presynaptic sites. Postsynaptically the peptide enhanced the response to neurotransmitters apparently without appreciably altering the properties of the resting membrane. In one preparation, similar amounts of ACTH_{4-10} augmented the depolarizing and hyperpolarizing responses to agonists for two distinctly different types of synaptic receptors. This nonspecific action, which allows the peptide to alter the input/output relationship in postsynaptic responses mediated by several types of synapses, would have little physiological meaning if all types of synapses were randomly activated. The nonspecific boosting of the postsynaptic response could become significant however, if a specific synaptic input dominated. The response to this input would be selectively enhanced in presence of the peptide. Presynaptically, ACTH_{4-10} may stimulate the release of neurotransmitter from the nerve terminals. Electrophysiological evidence of this action has so far been obtained only with cholinergic nerve fibers. However, according to biochemical data (for review see Versteeg, 1980) it is quite likely that ACTH_{4-10} also enhances release from other than cholinergic fibers. This mixed pre- and postsynaptic action of ACTH_{4-10} may help in understanding the variety of electrophysiological and behavioral effects (see for review de Wied and Jolles, 1982) of ACTH_{4-10} .

In addition, there is indirect electrophysiological evidence to suggest that the stimulus-induced release of acetylcholine from the nerve fibers innervating muscles is decreased in presence of ACTH. If this is the case it is another example of the various activities present in the ACTH molecule (for review see de Wied and Jolles, 1982).

5. EFFECTS OF ENDORPHINS AND ENKEPHALINS ON MACROELECTROPHYSIOLOGICAL MEASUREMENTS

5.1. ELECTROENCEPHALOGRAM

Animals studies with opiate-like peptides derived from the C-terminal portion of the β -LPH molecule showed both that these peptides are very potent in inducing epileptiform EEG activity when administered directly into the brain and that their effects are mediated through stereospecific opiate receptors. Urca *et al.* (1977) described alterations which developed in the frontal and occipital EEG following i.c.v. injection of methionine⁵-enkephalin (ME) in rats. Almost immediately after the injection of ME (200 μ g) the EEG changed into rapidly repeating spikes which evolved into slower, high amplitude sharp waves (4–6/sec) after 20–30 sec. This pattern was often followed by a desynchronized EEG of unusually low amplitude and by a second build-up of the repetitive sharp waves, spikes or wave complexes which lasted for about 20 sec. This activity was later replaced by a slow wave activity with spikes and polyspikes or complex waves occurring at a frequency of 5–7/sec. In that study, i.p. injected naloxone neither prevented nor reversed the ME-induced EEG seizures. However, in subsequent experiments (Frenk *et al.*, 1978a) in which 100 μ g leucine⁵-enkephalin (LE) was used, pretreatment of rats with naloxone greatly attenuated or blocked the development of the abnormal EEG activity.

The electroencephalographic changes induced by i.c.v. D-Ala²-ME (DAME) amide closely resembled those seen after i.c.v. administered morphine. The slow waves with bursts of high voltage spikes that occurred in the frontal and occipital EEGs following microinjections of 10 μ g DAME amide could be mimicked with 5 μ g of the opiate alkaloid. Morphine 10 μ g converted the EEG patterns into the same high voltage spike continuum as did 20 μ g of the peptide. Naloxone prevented the development of the peptide- and morphine-elicited EEG patterns (Tortella *et al.*, 1978). Microinjection of ME or LE into the dorsomedial thalamic nucleus, but not into the periaqueductal matter triggered epileptic-like activity similar to that seen following i.c.v. administration of these peptides (Frenk *et al.*, 1978b).

When centrally administered, β -endorphin (β E, β -LPH_{61–91}) appeared to induce EEG and behavioral seizures more potently even than the enkephalins. La Bella *et al.* (1978) showed that 2 μ g of this peptide injected into one of the lateral brain ventricles could convert the cortical EEG of rats into spike activity. In rabbits, clear paroxysmal high voltage discharges were observed only if a 60 μ mol solution of β E in artificial cerebrospinal fluid was injected into the lateral ventricles. Administration of the peptide in the third ventricle only slowed down the dominant background activity from 6–7 Hz to 3–5 Hz and markedly decreased the average power of the signal. These naloxone reversible effects were strongest 10–20 min after the injection and the EEG returned to control levels within 90 min (Firemark and Weitzman, 1979).

There is strong evidence to suggest that the epileptiform effects of the opioid peptides on the EEG, which are mediated by the opiate receptors, originate in the forebrain limbic structures and the hippocampus in particular. The marked paroxysmal activity with epileptic spikes seen in the cortical EEG shortly after a 3–5 μ M Ringer solution of synthetic porcine or human β E was i.c.v. injected, developed first and reached its highest amplitude in subcortical structures like the septum, medial preoptic area, central amygdala and the medial dorsal thalamus before it appeared on leads from the somatosensory motor cortices (Henriksen *et al.*, 1978). In the encephale isolé cat, even 0.2 μ g of LE administered directly into the hippocampus resulted in epileptiform discharges lasting several min, followed by a flat EEG. The hippocampus of rats anesthetized with urethane showed seizures only after

5 μ g of the peptide had been given (Elazar *et al.*, 1978). The great susceptibility of hippocampal tissue to epileptic discharges following exposure to opiate-like peptides was elegantly demonstrated by Taylor *et al.* (1979) in isolated hippocampus *in vivo*. These authors transplanted the embryonal regio superior (CA1 and CA2) of the fetal rat hippocampus into the anterior eye chamber of an adult rat. The transplants were allowed to develop for at least two months *in oculo*. For the experiments, a recording chamber was formed by means of a plastic ring around the transplant. Superfusion of the thus isolated hippocampus with 10^{-7} M β E resulted in naloxone reversible epileptic discharges.

Blockade of the inhibitory GABA-ergic neurotransmission is perhaps the principal mechanism for induction of hippocampal seizure activity by opiate-like peptides (Nicoll *et al.*, 1980; Dingledine, 1981). However, the blockade of GABA-ergic transmission is presumably only one of the many effects of opiate-like peptides on brain processes. Dopamine, acetylcholine, serotonin and even vasopressin release were also affected by these peptides (Versteeg, 1980; Iversen *et al.*, 1980; Bickell and Leng, 1982).

In a combined behavioral and electrophysiological study, de Wied *et al.* (1978a) examined the effects of i.c.v. administered β E on the extinction of pole jumping avoidance behavior and on the frequency of hippocampal theta activity in rats during PS. When 20–30 ng of the peptide was injected the extinction of pole jumping was delayed and the occurrence of high theta frequencies in hippocampal EEG activity was increased. There were no behavioral or electroencephalographic signs of a seizure type of activity in the peptide-treated animals. Met⁵-enkephalin (20 ng) also given i.c.v. did not affect hippocampal activity. The β E-induced frequency increase in hippocampal theta rhythm was also reported by Henriksen *et al.* (1978). Morphine suppressed the occurrence of theta rhythm (Linseman and Grupp, 1980; Klemm *et al.*, 1977) suggesting that the effect of β E on theta rhythm was presumably associated with an activity other than the opiate-like activity of β E. The non-opiate activity of β E was recently disclosed in behavioral experiments with the pole jumping avoidance response. In these studies β E, β -LPH_{61–69} and α E (β -LPH_{61–76}) all delayed extinction of the pole jumping avoidance response. Pretreatment of rats with naloxone in doses which prevent β E-induced analgesia did not interfere with the effect of α E on the extinction of the avoidance response (de Wied *et al.*, 1978a). Another peptide, γ E (β -LPH_{61–77}) had the opposite effect and facilitated the extinction of the pole jumping avoidance response. Removal of the N-terminal amino acid tyrosine from γ E and α E yielded the peptides (Des-Tyr¹)- γ E (β -LPH_{62–77}) and (Des-Tyr¹)- α E (β -LPH_{62–76}) that were devoid of most of the opiate-like analgesic activities. These peptides however retained the full activity of the original peptides in the avoidance procedures (de Wied *et al.*, 1978a; Bohus *et al.*, 1980).

The rat pituitary and rat brain (Loeber *et al.*, 1979; Verhoef *et al.*, 1980, 1982) contain DT γ E and DT α E. Depending on the pH, DT γ E and DT α E could be obtained preferentially *in vitro* from the β E molecule by exposure of the molecule to enzymes associated with an enriched synaptosomal plasma membrane fraction (Burbach *et al.*, 1980). It was therefore suggested that two peptides formed from the same parent molecule may have different functions in the brain. Recently, we have studied the effects of DT α E and DT γ E on the spontaneous EEG of the lateral septal complex (LSC) and frontal cortex of the rat (Urban and de Wied, 1982). As the activity of DT γ E in various behavioral tests resembled that of haloperidol (de Wied *et al.*, 1978b; Kovacs and de Wied, 1978; Van Ree *et al.*, 1980) this neuroleptic drug was also included in the study. The rats were injected on two subsequent days either with placebo (saline solution) or with one of the compounds to be tested. EEG activity was quantified by power spectral analysis. The effects of treatment were evaluated by computing the differences between average spectra of corresponding periods in the placebo and the peptide or haloperidol treatment sessions and comparing these differences in the 't'-test for paired values. The 't' profiles so obtained showed a significant increase in activity of the 5 Hz band following 2 μ g of DT α E. Treatment with 10 μ g of DT γ E had the opposite effect and led to a decrease in the amount of activity in this band. In addition, DT α E promoted the production of 15–20 Hz activity while DT γ E decreased the amount of 20–30 Hz activity. EEG activity exhibited a marked

variability which persisted throughout the recording sessions following administration of the peptides. Haloperidol markedly decreased the proportion of 10–15 Hz activity. The alterations in EEG of the frontal cortex followed those in LSC but were less pronounced. The differences in the time course and frequency bands affected suggest that the effects of peptides and haloperidol on the EEG activity of LSC are not mediated by the same mechanisms. However, the data support the notion that two closely related neuropeptides derived from the same molecule may have different functions in the brain.

There have been only few studies published concerning the effect of opiate-like peptides on the human EEG. In one of these studies (Von Graffenried *et al.*, 1978), 12 healthy volunteers were injected (i.m.) with 0.1–2 mg of a synthetic analog of ME (FK₃₃₋₈₂₄). Spectral analysis of the spontaneous EEG recorded following the treatment revealed a slowing down of the alpha-waves, an increase of almost 50% in beta activity and an increase in the total power of the signal. The increase in alpha band activity was detectable by visual inspection of the EEG records. This pattern was most pronounced 2–4 hr after administration of the peptide. Nalorphine injected 90 min after FK₃₃₋₈₂₄ failed to interfere with the peptide-induced changes. In another study on 20 volunteers, 0.5 mg FK₃₃₋₈₂₄ led to a naloxone reversible increase in alpha and beta band activities, perhaps as a result of hypersynchronized and spindling activities (Krebs and Roubicek, 1979). Angst *et al.* (1979) treated 6 female patients with 10 mg of synthetic β -endorphin given in a single i.v. injection. Visual examination of the spontaneous EEG recorded from the temporo-parietal and parieto-occipital leads showed no obvious alteration. However, spectral analysis of the data revealed an increase in power of the alpha (7.5–12.5 Hz) and beta (12.5–22.5 Hz) bands in the first 60 min after injection. This increase was later followed by a decrease of activity in these bands. Interestingly, none of these studies reported any development of the epileptoid EEG waves typical of the opiate-like activity of peptides.

5.2. EVOKED POTENTIALS

To our knowledge no studies have been published on the effects of EPs of peptides derived from the β -LPH molecule.

5.3. MULTIPLE-UNIT ACTIVITY

There is only one study (Urca *et al.*, 1977) which demonstrated an opiate-like effect of ME on MUA. In this study, the i.c.v. administration of a large (200 μ g) dose of ME increased the frequency of action potentials in MUA records from the periaqueductal central grey in the same way as did 30 μ g of morphine. The MUA increase could be prevented or completely reversed by naloxone.

6. EFFECTS OF ENKEPHALINS AND ENDORPHINS ON MICROELECTROPHYSIOLOGICAL MEASUREMENTS

6.1. SINGLE NEURONS *In Vivo*

The analgesic action of morphine and of other opiate alkaloids has been attributed in part to a selective depression by opiates of transmission between the primary afferent fibers and the nociceptive dorsal horn (DH) neurons (Henry and Newman, 1974; Calvillo *et al.*, 1974). It therefore seemed logical to hypothesize that the analgesia elicited by enkephalins and endorphins may have a similar mechanism, namely suppression of the pain-induced activity in the DH neurons. Such a hypothesis was supported by the finding of a high concentration of the stereospecific opiate receptors in the substantia gelatinosa, the site of terminal branches of the sensory afferents (Lamotte *et al.*, 1976; Pert *et al.*, 1975; Atweh and Kuhor, 1977a). In addition, histochemical experiments revealed the presence of immunoreactive enkephalin-like peptides in many axons and cell bodies in this region (Elde *et al.*, 1976; Hökfelt *et al.*, 1977a,b) suggesting a physiological role for enkephalins in the transmission of pain stimuli. The earlier iontophoretic experiments indeed showed that ME and LE, similarly to morphine, levorphanol but not dextrophan, an inactive D-enantiomer of levorphanol, inhibit spontaneous or stimulated activity in most of the DH neurons

(Duggan *et al.*, 1976; Zieglgänsberger and Bayerl, 1976b; Duggan and Headley, 1978). The depressant action of opioid peptides, like that of opiate alkaloids, could in many cases be reversibly abolished by iontophoretic or systemic administration of naloxone indicating the involvement of stereospecific opiate receptors in the response of DH to enkephalins. The caudal trigeminal nucleus is functionally equivalent to the DH of the spinal cord. Here too, the locally applied ME or β -E caused a naloxone reversible depression in spontaneous firing rate and decreased the trigeminal nucleus neurons to pain-inducing stimuli (Andersen *et al.*, 1978; Henry, 1978). Subsequent experiments revealed the more general character of the depressant action exerted by opioid peptides on mammalian neurons (see Table 1). Thus, met-enkephalins and endorphins diminished the spontaneous and stimulus-induced neuronal responses not only in the medulla oblongata, brain stem and thalamus, i.e. in structures transmitting the pain stimuli to the higher brain centra, but also in structures like the hypothalamus, striatum and frontal cortex that are remote from the pathways mediating the noxious stimuli.

In contrast to the DH cells, the spinal cord interneurons identified by electrical stimulation of ventral roots as the Renshaw cells, were excited by ME and morphine. In addition, ME decreased the reactivity to acetylcholine of these presumably cholinceptive

TABLE 1.

Animal/ Structure	Type of Neuron	Peptide	Dominant Effect	Antagonist Reversible	Reference
Cat spinal cord	nociceptive and non-nociceptive neurons	ME ME-amide	applied to SUGEL ↓ noxious S-IA little effect on non-noxious S-IA	+	Duggan <i>et al.</i> , 1976
Cat spinal cord	DH neurons	ME ME-amide	near cell bodies ↓ SA and S-IA in SUGEL ↓ SA ↓ noxious S-IA little or no effect on non-noxious S-IA	+	Duggan <i>et al.</i> , 1977
Cat spinal cord	DH neurons	ME LE	no effect on SA S-IA		Davies and Dray, 1978
Cat spinal cord	DH neurons	D-Ala ² LE-amide	↓ noxious S-IA no effect on non- noxious S-IA	+	Davies and Dray, 1980
Cat spinal cord	DH neurons	ME-amide	applied to SUGEL ↓ noxious S-IA	+	Duggan and Headley, 1978
Cat spinal cord	nociceptive and other neurons	ME	↓ SA ↓ S-IA ↓ glutamate-IA ↓ Substance P-IA	+	Zieglgänsberger and Tulloch, 1979a,b.
Cat spinal cord	nociceptive neurons	ME	↓ noxious S-IA	not tested	Randic and Miletic, 1978
Cat spinal cord	nociceptive neurons	β E	↓ noxious S-IA	not tested	Henry, 1978
Cat spinal cord	Renshaw cells	ME LE	↑ SA	+	Davies and Dray, 1976a,b
Cat spinal cord	Renshaw cells	ME LE	↑ SA	+	Davies and Dray, 1978
Cat spinal cord	DH neurons	ME	↓ SA	not tested	Miletic and Randic, 1982
Cat caudal trigeminal nucleus	nociceptive and non-nociceptive neurons	ME	↑ SA in nociceptive neurons ↓ noxious S-IA in nociceptive neurons little or no effect on non-nociceptive cells	+	Andersen <i>et al.</i> , 1978
Rat medulla oblongata	unidentified neurons	ME	↓ SA	not tested	Hill <i>et al.</i> , 1976.

TABLE 1 *cont.*

Animal/ Structure	Type of Neuron	Peptide	Dominant Effect	Antagonist Reversible	Reference
Rat medulla oblongata	respiratory neurons	ME	↓ SA	+	Denavit-Saubié <i>et al.</i> , 1978
Cat brain stem	n. reticularis giganto-cellularis, n. pontis caudalis neurons	ME LE βE	↓ SA ↓ Homocysteate-IA ↓ noxious S-IA	—	Gent and Wolstencroft, 1976a,b; Gent <i>et al.</i> , 1977
Cat brain stem	n. reticularis giganto cellularis, n. pontic caudalis, n. raphe bulbaris and periaqueductal gray nociceptive neurons	ME LE αE βE	↓ SA ↓ noxious SA	—	Wolstencroft <i>et al.</i> , 1978
Cat brain stem	unidentified neurons	ME	↓ SA	+	Davies and Dray, 1978
Rat brain stem	unidentified neurons	ME	↓ SA ↑ SA	+	Bradley <i>et al.</i> , 1976
Rat brain stem	n. reticularis giganto-cellularis neurons	ME LE D-Ala ² -ME- amide D-Ala ² -D- Leu ⁵ -E	↓ SA	+	Bradley <i>et al.</i> , 1978a,b
Rat brain stem	n. reticularis giganto-cellularis and n. pontis	ME βE	↓ SA	+	Bradley and Bramwell, 1977
Rat brain stem	n. reticularis lateralis	ME LE αE βE	↓ SA	+	Nicoll <i>et al.</i> , 1977 Siggins <i>et al.</i> , 1978
Rat brain stem	nociceptive neurons	ME	↓ noxious S-IA	+	Hosford and Haigler, 1980
Rat brain stem	periaqueductal gray neurons	ME	↓ SA	+	Frederikson and Norris, 1976
Rat brain stem neurons	locus coeruleus	ME	↓ SA	+	Young <i>et al.</i> , 1977
Rat brain stem	locus coeruleus neurons	ME	↓ SA	not tested	Guyenet and Aghajanian, 1977
Rat thalamus	nociceptive neurons	ME	↓ noxious S-IA	not tested	Hill <i>et al.</i> , 1976
Rat thalamus	nociceptive neurons	ME	↓ noxious S-IA	— (+)	Hill and Pepper, 1976
Rat thalamus	nociceptive neurons	D-Ala ² -D- Leu ⁵ -E D-Tyr ¹ -D- Ala ² -D-Leu ⁵ - E	↓ noxious S-IA no effect	+	Hill and Pepper, 1978a,b
Rat thalamus	unidentified neurons	ME LE αE βE	↓ SA	+	Nicoll <i>et al.</i> , 1977 Siggins <i>et al.</i> , 1978
Rat caudate nucleus	unidentified neurons	ME LE αE βE	↓ SA	+	Nicoll <i>et al.</i> , 1977
Rat caudate nucleus	unidentified neurons	ME	↓ SA	+	Frederikson and Norris, 1976
Rat striatum	unidentified neurons	ME	↓ SA	+	Fry <i>et al.</i> , 1978

DH, dorsal horn; SA, spontaneous activity; S-IA, stimulus-induced activity; SUGEL, substansia gelatinosa; ↑, increase; ↓, decrease.

neurons and in that action the peptide differed from morphine which enhanced the acetylcholine-induced excitation of the Renshaw cells (Davis and Dray, 1976a,b). The hippocampal pyramidal neurons were another kind of cells which was repeatedly excited by opioid peptides (Nicoll *et al.*, 1977). Recently, Zieglgänsberger *et al.* (1979) obtained simultaneous recordings from a hippocampal pyramidal neuron and from another cell that could have been an interneuron because the increased firing in the interneuron was time-related to inhibition of the pyramidal cell. β E applied by pressure ejection in the vicinity of these cells markedly attenuated the spontaneous activity of the interneuron and at the same time induced firing in the pyramidal cell. Iontophoretically released Mg^{2+} or bicuculine suppressed the β E-induced excitation, suggesting that in exciting the pyramidal cells, the opioid peptides presumably acted upon the inhibitory interneurons and suppressed the activity in these neurons.

Some amino acids, e.g. L-glutamate or aspartate elicit powerful excitation in most of the mammalian neurons (Curtis *et al.*, 1972). These amino acids are therefore frequently employed as a tool to excite the silent or the slowly spontaneously firing neurons particularly in those microiontophoretic experiments in which the actions of presumed inhibitory compounds on single neurons *in vivo* are being examined.

ME-amide, *N*-acetyl-D-Ala²-Lys-ME, ME, D-Ala²-ME or β E markedly attenuated the neuronal responses of neurons to glutamate and aspartate (Frederikson and Noris, 1976; Zieglgänsberger *et al.*, 1976; Segal 1977; Zieglgänsberger and Tulloch, 1979a,b). In addition, enkephalin and analogs also appeared to depress the excitation of the spinal cord and neocortical neurons by acetylcholine (Davies and Dray, 1976a; Segal, 1977; Palmer *et al.*, 1978) just as they diminished the increase in firing rate elicited in some spinal cord cells by iontophoretic application of Substance P (Zieglgänsberger and Tulloch, 1979b). Unfortunately, we do not know whether the opioid peptides can elicit all these different effects in the same neurons or whether they exert different effects in different neurons.

Many effects of enkephalins and endorphins on single neurons *in vivo* could be prevented or reversed by administering the stereospecific opiate receptor antagonist naloxone (see Table 1) and thus they are presumably mediated by some kind of opiate receptor. Hosford and Haigler (1980) compared the effect exerted by ME and morphine on the stimulus-induced response in the same mesencephalic reticular neuron of rats. Both compounds affected the response of these neurons in one of the three following ways. ME but not morphine blocked the stimulus-induced excitation, morphine and not ME abolished the response, both morphine and ME suppressed the firing evoked in the reticular neurons by nociceptive stimulation. Naloxone either given systemically or applied iontophoresis antagonised the action of ME as readily as it blocked the effects of opiate alkaloids. This suggests that at least two kinds of stereospecific opiate receptors may exist on the same neuron. The existence of different (μ and δ) opiate receptors in the spinal cord and brain had been expected from receptor binding studies (Lord *et al.*, 1977; Fields *et al.*, 1980). Electrophysiological evidence that μ - and δ -types of opiate receptors coexist on the same anterior neurons has recently been provided by Egan and North (1981). The functional significance of multiple opiate receptors on the same cells is not known.

Naloxone was not always equally effective in antagonizing the action of opioid peptides on single neurons *in vivo*. The antagonist given i.v. abolished the ME-induced decrease in reactivity of the thalamic neurons to pain stimuli in only 2 of 8 cells tested (Hill *et al.*, 1976). Gent and Wolstencroft (1976a,b) were unsuccessful in antagonizing the inhibitory action of enkephalins on the raphe, periaqueductal gray and brain stem neurons in cats. Fry *et al.* (1979) reported that most of the excitatory and inhibitory responses of hippocampal neurons to iontophoretically applied ME were resistant to pretreatment with naloxone. Thus, it cannot be excluded that the opioid peptides may also have some action other than the stereospecific actions on single cells.

6.2. EXPERIMENTS *IN VITRO*

The significant amount of stereospecific opiate receptors that autoradiographic and

other methods detected in the substantia gelatinosa of the spinal cord, or elsewhere within the brain, appears to be located on the presynaptic fibers (Atweh *et al.*, 1978; Lamotte *et al.*, 1976; Fields *et al.*, 1980). It could therefore be expected that opioid peptides and opiate alkaloids act at least partly at the presynaptic sites. Indeed, enkephalins and their analogs significantly reduced the amounts of acetylcholine, noradrenaline, dopamine or Substance P released from various neural tissues by K^+ or by electrical stimuli (Kosterlitz and Hughes, 1975; Waterfield and Kosterlitz, 1975; Loh *et al.*, 1976; Jessel and Iversen, 1977; Subramanian *et al.*, 1977; Waterfield *et al.*, 1977; Mudge *et al.*, 1979a,b). By separating the pars nervosa/pars intermedia from the hypothalamus of rats, Iversen and his co-workers (Iversen *et al.*, 1980) obtained a preparation which contained only the neurosecretory axons and their terminals but not the cell bodies of the hypothalamic neurosecretory neurons, the site of synthesis of vasopressin. It had been shown previously with this preparation that electrical stimuli delivered to the distal part of the pituitary stalk released vasopressin into the incubating medium (Dreifuss *et al.*, 1971). Incubating the posterior pituitary fragments with D-Ala²-D-Leu⁵-enkephalin (DADL) led to a marked depression of the electrically elicited release of vasopressin. The efflux of luteinizing hormone-releasing factor and somatostatin from hypothalamic slices challenged by K^+ could also be suppressed by opioid peptides (Drouva *et al.*, 1981). The opiate receptor agonist etorphine decreased the amplitude of the excitatory postsynaptic potentials elicited in cultured murine spinal cord cells. Statistical analysis of these potentials indicated that the release of transmitter mediating the EPSPs had been suppressed (MacDonald and Nelson, 1978). In other experiments on cats (Carstens *et al.*, 1979), systemically administered morphine elevated the threshold for antidromic excitation of the small diameter C-primary afferents. Thus, the presynaptic depression of neurotransmitter and hormone release may be an important mechanism in the action of opioid peptides on brain and neuroendocrine processes.

The spinal cord primary afferents are some of the few presynaptic fibers that are accessible to electrophysiological measurements. Stimulating the ventral or the adjacent dorsal roots elicits depolarization of these afferents that can be measured in the proximal ends of the dorsal roots (DR) as a transient voltage deflection called dorsal root potential (DRP). Functionally, the depolarization of primary afferents indicates the presynaptic inhibition of transmitter release from the primary afferents and the magnitude of the DRP reflects the amount of this inhibition (for review see Schmidt, 1971). Superfusion of the isolated frog spinal cord with Ringer solution containing $10\text{ }\mu\text{M}$ of D-Ala²-ME (DALA) almost completely abolished the DRPs in response to ventral roots stimulation. The depression by opioid peptides of the DRPs was also found by others in rats and cats (Sastri, 1978; Pomeranz and Gurevich, 1979; Suze and Jessel, 1980). According to the current concept (Schmidt, 1971; Burke and Rudomin, 1977), the presynaptic inhibition in the primary afferents is caused by depolarization of these afferents with GABA released synaptically in the vicinity of these terminals. A decrease in magnitude of DRPs would therefore indicate a diminished release of GABA or attenuated effect of the inhibitory transmitter on the primary afferents. The former seems more likely since ME increased rather than decreased the GABA-induced depolarization in the DR ganglion cells (Williams and Zieglgänsberger, 1981). The slow IPSP elicited transsynaptically in the frog SG neurons markedly decreased in magnitude during perfusion of SG with 10^{-7} M ME or D-Ala²-ME (Wouters and van den Bercken, 1979b, 1980). This effect was presumably also a presynaptic one as the postsynaptic hyperpolarization of the neurons in response to the presumed mediator of the IPLSPs was little affected by peptides.

During sucrose gap measurements of the DRPs in frog spinal cord, Nicoll and collaborators (Nicoll *et al.*, 1980) noticed a small but consistent hyperpolarization of the primary afferent fibers exposed to DALA. The peptide-elicited hyperpolarization could not be abolished by adding the synaptic transmission blocking agents tetrodotoxin (TTX) or Mg^{2+} into the incubating medium and thus it must have reflected a direct action of DALA on the fibers. Interestingly, the dorsal root ganglionic neurons often used as model for the presynaptic membranes of the primary afferents were not hyperpolarized by enkephalins

and their analogs (Mudge *et al.*, 1979a,b; Williams and Zieglgänsberger, 1981). It is not clear how the hyperpolarization of terminals could lead to decreased transmitter release from these terminals. The model of presynaptic inhibition in primary afferents assumes that the GABA-induced depolarization of afferent fibers will diminish the amplitude of arriving action potentials by shunting the membrane currents and thus prevent depolarization of the terminals to the extent necessary for normal functioning of the release mechanisms. From this it follows that hyperpolarizing the presynaptic fibers will increase rather than decrease the amplitude of an action potential invading the terminal and this should increase the amounts of mediator released from the terminal. The hyperpolarization associated with increased Cl^- conductance (Kawai and Niwa, 1978) could however bring about the same shunting effects as depolarization. Conceivably, in diminishing transmitter release from presynaptic terminals the opioid peptides could have interfered with other presynaptic processes, e.g. calcium influx. The action potential of DR ganglionic cells was shortened in presence of 10^{-7} M D-Ala²-ME-amide (Mudge *et al.*, 1979a,b). Eliminating the Na^+ component in the inward currents with TTX did not abolish the peptide effect, thus indicating that the enkephalin analog diminished the Ca^{2+} inward current. The post-synaptic dendrite Ca^{2+} spike in hippocampal pyramidal cells was however not affected by enkephalins (Haas and Gähwiler, 1981).

Most of the intracellular recordings that could be obtained from neurons in the spinal cord and many other brain structures showed that the opioid peptides failed to alter the RMP and membrane resistance (MR). Thus, the cat DH cells and motoneurons exhibited no changes in RMP and MR following iontophoretic application of ME and LE (Zieglgänsberger and Tulloch, 1979a). Similar measurements remained unchanged during superfusion of rat and chicken embryos with 1–20 μM concentrations of ME, D-Ala²-D-Leu⁵-enkephalin or D-Ala²-ME amide (Mudge *et al.*, 1979a; Williams and Zieglgänsberger, 1981). ME and LE never altered the RMP of the myenteric plexus neurons isolated from guinea-pig ileum when the peptides were applied iontophoretically in the vicinity of these neurons (North *et al.*, 1979). The membrane potential, input resistance and spike threshold of the hippocampal pyramidal cells in slice preparations (Nicoll *et al.*, 1980; Dingledine, 1981; Siggins and Zieglgänsberger, 1981) or maintained in tissue culture (Gähwiler, 1980) could not be changed by either iontophoretically released or superfused ME, LE, D-Ala²-D-Leu⁵-enkephalin, the enkephalin analogs FK 33–824, FW 34–569 or βE .

The marked ineffectiveness of the opioid peptides on electrical properties of the resting cell membranes in CNS neurons does not fully justify the exclusion of these principles from the list of candidates for neurotransmitters. In cultures to fetal murine spinal cord cells Barker (Barker *et al.*, 1978b, 1980) found several transmitter-like actions of ME and LE including a presumptive increase in Cl^- conductance and rapidly desensitizing excitation. In addition, the peptides altered the spike threshold of the neurons in the absence of, or independent of any other membrane effects. It could be argued that those actions of peptides were atypical, perhaps limited to the immature spinal cord neurons. However, North (North, 1979; North *et al.*, 1979) observed repeatedly that guinea-pig myenteric plexus neurons were hyperpolarized when enkephalins were administered by superfusion. The naloxone reversible hyperpolarization of myenteric cells had never occurred following iontophoretic release of the peptides near the cell bodies. Considering the morphology of the plexus cells, North (1979) interpreted these findings as being the result of peptide action on cell processes remote from the cell bodies. Conceivably, alterations in the resting membrane potential or membrane conductance elicited in the cell processes are not always reflected in the intracellular measurements made at the soma membrane. An action remote from the cell body may perhaps explain the naloxone reversible hyperpolarization and increased membrane conductance that could be found in some but not all neurons of locus coeruleus slices following superfusion with 300 nM concentrations of D-Ala²-ME or D-Ala²-D-Leu⁵-enkephalin (Pepper and Henderson, 1980). Thus, a transmitter-like action of opioid peptides on the postsynaptic membrane is quite possible, at least in some cases. In frog SG cells, ME and D-Ala²-ME increased the negativity of the potential recorded

across the sucrose gap (Wouters and van den Bercken, 1979b, 1980). In presence of a synaptic activity blocking agent, a treatment-related increase in negativity would indicate hyperpolarization of the SG neurons. Synaptic activity in the ganglion was not abolished and thus some presynaptic mechanism in this effect of enkephalins cannot be excluded.

It has frequently been observed that the response of single neurons to iontophoretically applied L-glutamate or aspartate is considerably reduced in presence of opioid peptides. Such a naloxone reversible depression by opioids of amino acid-induced excitation was seen for example in spinal cord moto- and interneurons (Zieglgänsberger and Tulloch, 1979a,b), hippocampal pyramidal neurons (Segal, 1977) and in cortical neurons (Zieglgänsberger and Fry, 1976; Zieglgänsberger *et al.*, 1976). It has been shown that the neuronal excitations elicited by L-glutamate were associated with depolarization (see e.g. Zieglgänsberger and Puil, 1973; Zieglgänsberger and Champagnat, 1979; Gähwiler, 1980; for review see Krnjevic, 1974) and with markedly enhanced conductance for the sodium ions (Zieglgänsberger and Puil, 1973). In contrast to voltage-dependent (depolarization-induced) Na^+ conductance, the glutamate-induced increased permeability for sodium was not affected by TTX. It may therefore be assumed that as, e.g. in the case of acetylcholine at the muscle fiber end plate (Katz, 1966), the glutamate-induced drop in membrane resistance involves primarily activation of the receptor-coupled ionophores. It appeared from the analysis of the reciprocal membrane currents as a function of the glutamate pulse magnitude that LE altered neither the number of glutamate molecules involved in the postsynaptic response nor the affinity of the receptors for this amino acid (Barker *et al.*, 1978a). As mentioned earlier, the opioid peptides had little effect on the resting neuronal membrane indicating that they themselves do not activate any specific-coupled ionic conductances. However, once Na^+ conductance had been activated by an appropriate agonist, the functioning of the receptor-coupled sodium channels presumably became different in presence of the peptide. The modulatory effect of opioid peptides upon receptor-coupled Na^+ conductance certainly deserves greater attention as it may turn out to be an important postsynaptic action of these peptides. Perhaps this or a similar mechanism may also be involved in the peptide-induced attenuation of the excitation elicited by acetylcholine (Davies and Dray, 1976) or by Substance P (Zieglgänsberger and Tulloch, 1979a).

7. CONCLUDING REMARKS

The electrophysiological experiments reviewed here provide convincing evidence that the peptides derived from pro-opiomelanocortin may act upon the brain and alter neuronal processes. The experiments thereby support the original hypothesis (de Wied, 1969) indicating the brain as a target for pituitary hormones and for the neuropeptides formed from these hormones. The actual role of these principles in the neurophysiology of the brain has however not been demonstrated by these experiments. Peptides released from the pituitary gland can reach their sites of actions within the brain by way of the general circulation, by retrograde blood flow through the hypophyseal portal system or by means of the cerebrospinal fluid (Oliver *et al.*, 1977; Dorsa *et al.*, 1979; Mezey *et al.*, 1978; Raporot *et al.*, 1980). It can therefore be reasoned that the peptides administered systematically or i.c.v. will be carried and distributed within the brain by the same mechanisms as the principles released from the pituitary. It became clear from several experiments that peptides given via these routes can alter ongoing neuronal activity in many parts of the brain. For example, the systemic or i.c.v. injection of ACTH_{4-10} and the closely related α -MSH accelerated the hippocampal theta activity accompanying PS (Urban and de Wied, 1976), slowed down the same activity recorded during operant conditioning (Urban *et al.*, 1974) and altered EEGs and ERs derived from different cortical areas in humans and animals (Sandman *et al.*, 1971; Velasco *et al.*, 1972; Braconier *et al.*, 1979; Miller *et al.*, 1981). Interestingly, ACTH_{4-10} did not elicit activity in single neurons (Gent and Normanton, 1980) just as it failed to induce hippocampal theta activity or any other pattern of EEG activity indicating the activation of a specific neuronal network.

However, ACTH₄₋₁₀ considerably changed the responsiveness of single neurons and neuronal networks when they had been activated by some kind of stimulus (Urban and de Wied, 1976, 1978; Wouters and van den Bercken, 1979a). These effects mostly became visible some 30 min following the administration of ACTH₄₋₁₀, reached a maximum between 60–90 min and lasted for several hours thereafter. A gradual long-term alteration in input/relationship of neuronal networks was not an action unique to ACTH₄₋₁₀. A number of structurally different peptides e.g. β E arginine⁸-vasopressin, Des-glycinamide arginine⁸-vasopressin or oxytocin all exhibited this type of effect following systemic or i.c.v. administration (Urban and de Wied, 1975; Bohus *et al.*, 1978; de Wied *et al.*, 1978a; Urban and de Wied, 1978). Thus if the pituitary releases peptides that are predetermined to act within the brain, it presumably does so in order to exert this kind of modulatory action.

It has been proposed that the centrally acting peptides of pituitary origin stimulate the acquisition and maintenance of adaptive responses to novel situations (de Wied, 1978). Conceivably, there can be as many adaptive responses as novel situations. The organism has no way of anticipating which set(s) of neurons, brain structures and neuronal networks it will need to engage in order to elicit a response appropriate to the novel situation. The releasing peptides to the brain which themselves do not constitute any direct stimulus for brain neurons, but which temporarily alter the responsiveness of these neurons to stimuli impinging upon them, would certainly be an excellent mechanism for improving the function in any set(s) of neurons, structures and neuronal networks needed for elaborating a response adequate to the novelty. The hypophyseal pro-opiomelanocortin is a precursor of β -LPH, ACTH, α -MSH, β -MSH, β E and many other peptides (for review see de Wied and Jolles, 1982). It is not yet known which of these peptides eventually enter the brain, and what amounts of the peptides need to be accumulated in this organ in order to perform some function. In addition, different kinds of neurons and transmitter systems may exhibit different sensitivities towards different peptides. Future experiments with the pituitary principles should therefore not only examine the action of different peptides on each electrophysiological parameter, but do so for each peptide at various doses (dose-response procedure).

In contrast to the pituitary peptides, the neuropeptides synthesized in neurons might act strictly locally and thus function as some kind of neurotransmitters. For many years it has been known that the neurohypophyseal hormones oxytocin (OX) and vasopressin (AVP) are synthesized in neurosecretory neurons of the paraventricular, supraoptic and supra-chiasmatic nuclei and transported by axons to the posterior pituitary (for review see Bern, 1966; Bern and Knowles, 1966; Hayward, 1977). The release of OX and AVP from their pituitary terminals is initiated by an action potential sent down the axon by the neurosecretory cells themselves (Wakerly and Lincoln, 1973; Cross, 1974; Wakerly *et al.*, 1978) and is also regulated by a depolarization-coupled, Ca²⁺-dependent mechanism (Dreifuss *et al.*, 1971) as is e.g. the release of neurotransmitter acetylcholine (Katz, 1966). Not all fibers of the hypothalamic neurosecretory neurons follow the common final pathway to the posterior pituitary. Some turn and project to several extrahypothalamic structures, e.g. the lateral septum (Buijs, 1978; Sofroniew and Weidl, 1978; Buijs *et al.*, 1980; Hoorneman and Buijs, 1982), forming synaptoid contacts with the LSC neuron (Buijs and Swaab, 1979). The microiontophoretically applied AVP and OX can act directly and excite the LS neurons (Joëls and Urban, 1982). If the action potentials invade and depolarize the LS neurosecretory terminals, this depolarization would presumably also be associated with the release of OX or AVP from these terminals (Buijs and van Heerikhuizen, 1982) and the released hormones could act in the same way as excitatory neurotransmitters. The mediobasal hypothalamic pro-opiomelanocortin of the β E system resembles in some aspects that of OX and AVP. Cell bodies synthesize a peptide, presumably pro-opiomelanocortin (Watson *et al.*, 1978; Bloch, 1979; Bugnon *et al.*, 1979; Sofroniew, 1979) and the fibers carry peptide material, presumably β E, to terminals in septal preoptic, subthalamic, thalamic and midbrain areas (Bloom *et al.*, 1978; Finley *et al.*, 1980). The spinal cord and brain neurons respond readily to β E ejected with pressure or by microiontophoresis in the vicinity of these neurons (for references see Table 1). The

neuronal responses to thus applied β E exhibit a short onset latency and stop shortly after the end of administration indicating the presence of mechanisms terminating the action of the peptide. There is *in vitro* evidence for membrane bound enzymes cleaving β E into γ , α and other shorter endorphins (Burbach *et al.*, 1980). Those or similar enzymes could be part of a cellular 'machinery' metabolizing β E and thus terminating its action. If the pro-opiomelanocortin cells were capable of generating an action potential that is propagated along the fibers, depolarizing the terminal and releasing β E from this terminal they could use this peptide in the same way as other neurons use neurotransmitters.

However *in vitro*, pro-opiomelanocortin peptides, including the enkephalins, appeared quite ineffective to alter the resting membrane potential and membrane resistance as do classical neurotransmitters (Katz, 1966). Instead, in many preparations, the peptides interfered with presynaptic transmitter release and with postsynaptic responses to transynaptic and chemical stimuli. This action may represent a novel kind of interneuronal communication or a mechanism regulating the homeostasis of neuronal processes in discrete brain loci.

The cleavage products of β -endorphin, the γ - and α -type endorphins, are not biologically inert peptides. When given s.c. or i.c.v. they elicited distinctly different effects on behavioral (Bohus *et al.*, 1978, 1980; de Wied *et al.*, 1978a) and electrophysiological (Urban and de Wied, 1982) parameters. It was therefore suggested that the synaptically released β E is transformed into γ - or α -type endorphins each having a different function at the site where it is formed (de Wied and Jolles, 1982). The biotransformation of β E into γ - and α -endorphin presumably occurs *in vivo* as both types of endorphins were found in small but detectable amounts in extracts prepared from septum, thalamus and midbrain tissue (Verhoef *et al.*, 1980, 1982). The endorphins recovered from the brain extracts could however have originated from the intrasynaptic compartment suggesting that enzymatic biotransformation of β E could occur in the terminals prior to secretion. In such a case, the effective end product released from the terminal would not be β E but some other peptide. Practically nothing is known about the action of β E and its metabolites on neurons and neuronal processes in structures indicated by immunohistochemistry as being recipients of β E peptidergic innervation. Only future electrophysiological experiments could give us a better insight into the significance of pro-opiomelanocortin and other peptidergic systems within the brain.

Finally, some neuropeptides derived from pro-opiomelanocortin may turn out to be new agents suited for the treatment of mental disorders (de Wied and Van Ree, 1982). For example, behavioral and electrophysiological experiments indicated a mildly excitatory action of ACTH₄₋₁₀ on the brain (see for review de Wied and Jolles, 1982 and also Sections 3.0 and 4.0 of this review). This property of the peptide could be applied e.g. to improve the brain function of elderly patients. In one clinical study, ACTH₄₋₁₀ weakly improved attention in mentally retarded subjects, facilitated retrieval from memory and reduced depression and confusion in elderly patients (Braconnier *et al.*, 1979). The orally acting ACTH₄₋₉ (Grevén and de Wied, 1977) (Org 2766) promoted friendliness, decreased unsociability, self-rated anxiety and depression and increased the self-rated competence in aged subjects (Pigache and Rigter, 1981). Another peptide, DT γ E, temporarily ameliorated the psychotic symptoms in a group of patients for whom treatment with neuroleptics was indicated (Verhoeven *et al.*, 1979). The neuroleptic-like activity of this peptide had been anticipated from the results of behavioral experiments utilizing avoidance procedures, various grip tests and self-stimulation (de Wied *et al.*, 1978b, 1980; Dorsa *et al.*, 1979b; van Ree and Otte, 1980). DT γ E differed from neuroleptics in some of its actions. The peptides did not displace [³H]spiperone, [³H]haloperidol and [³H]apomorphine from their specific binding to brain membrane preparations (Van Ree *et al.*, 1978) and elicited in the lateral septum of rats EEG patterns that were different from those seen after haloperidol administration (Urban and de Wied, 1982). In addition, the γ -endorphins failed to decrease locomotor activity and elicited neither sedation nor any other symptom indicating an action on the extrapyramidal systems that is intrinsic to neuroleptic drugs. Thus, the behavioral, biochemical and electrophysiological experiments suggested that the beneficial

therapeutic effects of neuroleptics presumably reside in actions other than those previously anticipated for agents of this group. The experiments also indicated that this kind of multidisciplinary effort is an efficient strategy for the development of new, more specific neurotropic drugs and an important approach that could provide new clues to the etiology of mental disorders.

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