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INTERACTION OF PEPTIDES RELATED TO ACTH, MSH AND β -LPH WITH NEUROTRANSMITTERS IN THE BRAIN

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1. INTRODUCTION

This review covers the literature on biochemical effects of peptides which are considered to be derived from pro-opiocortin (see Miller 1980; De Kloet *et al.*, 1980), i.e. ACTH, α -MSH and β -LPH and bioactive fragments of these three peptides, on the so-called classical neurotransmitters in the brain, *viz.* the catecholamines, acetylcholine, serotonin and γ -amino butyric acid (GABA). When overviewing the progress that has been made in the past decade, it becomes evident that two factors have had a crucial influence on the development of the strategies in this field of research. The first of these factors, which can be considered to have been of major importance, is the discovery of the existence in the mammalian central nervous system of peptide-containing neuronal systems, starting in the years 1974 and 1975 (for a recent review see: Hökfelt *et al.*, 1980). In particular the discovery, isolation and characterization of Methionine⁵-enkephalin (Met-enkephalin; β -LPH₆₁₋₆₅) and β -endorphin (β -LPH₆₁₋₉₁) (Hughes 1975; Hughes *et al.*, 1975; Simantov and Snyder 1976; Teschemacher *et al.*, 1975; Li and Chung 1976; Bradbury *et al.*, 1976; for a more complete bibliography see: Miller 1980; De Kloet *et al.*, 1980) have initiated an explosive expansion of the research concerning these endogenous opiate-like peptides, the results of which have contributed to the gradual development of the hypothesis that these and a variety of other peptides occurring in the brain within distinct neuronal systems, by acting as neuroregulators, are of essential importance for the homeostasis.

Neurochemical studies with ACTH, α -MSH and related peptides, it should be noted, have been carried out well before the discovery of neuronal systems in the brain containing these peptides. This can be ascribed mainly to the pioneering work of De Wied, which started in the early sixties and has led to the concept that ACTH and ACTH-analogs which are devoid of the classical endocrine effects of ACTH, like ACTH₁₋₁₀, ACTH₄₋₁₀ and Met(O₂)⁴,D-Lys⁸,Phe⁹ACTH₄₋₉ (ORG2766), exert their effects via an interaction with the brain (for reviews see: De Wied 1966; 1969; De Wied *et al.*, 1972; De Kloet and De Wied 1980; Bohus 1980; Van Nispen and Greven 1980). On basis of this concept a number of neurochemical studies have been carried out, mainly with ACTH₄₋₁₀, which inhibits the extinction of active avoidance behavior, and its (D-Phe⁷)-analog, which has an effect on the extinction of active avoidance behavior opposite to that of ACTH₄₋₁₀, rather than with ACTH₁₋₃₉ or ACTH₁₋₂₄, which, in addition to their behavioral effects, have full adrenocorticotrophic activity (Van Nispen and Greven 1980). In most of these studies the peptides were administered systemically, which also was the route of administration in most of the behavioral studies (De Kloet and De Wied 1980; Bohus 1980). This is in contrast to the studies with the opioid peptides, which both in behavioral and in biochemical experiments are administered intracerebrally (see Sections 2, 3 and 4).

Another factor of importance, by its nature, has had, and in fact still has, a marked influence on the direction of the research on the effects of neuropeptides on brain neuro-

transmitters. Here I am referring to the fact that the methodological developments and, hence, the possibilities to carry out adequate neurochemical experiments, have been very much dissimilar for the various classical neurotransmitters in the brain in the past decade. In the catecholamine field, for example, neuroanatomical research, primarily due to the availability of relatively specific and sensitive methods, has a long tradition. Moreover, for the catecholamines relatively adequate neurochemical methods have been available for a much longer time than has been the case for the other neurotransmitters. It therefore is logical that catecholamine research and, consequently, also the study of the possible interaction of neuropeptides with brain catecholamines has benefitted by this combination. Finally, the development of sensitive radiochemical methods for the measurement of neurotransmitters, their metabolites and the enzymes involved in their synthesis, has enabled researchers to go down in their experiments from whole brain or large brain parts to small brain parts and even individual brain nuclei. This has provided interesting new possibilities for approaching the question of how peptides interact with brain neurotransmitters at a high level of sensitivity.

The notion that peptides in the central nervous system are operating as neuroregulators, i.e. are participating in the functioning of the brain as neurotransmitters, neuromodulators or neurohormones (Barchas *et al.*, 1978; Barker 1978; Barker and Smith 1979) is supported by evidence coming from a variety of disciplines. In addition to (1) the immunocyto- and histochemical and radioimmunochemical data concerning their distribution and localization in the central nervous system and their occurrence in neuronal fiber systems, there are (2) the accumulating evidence, at least for some of these peptides, that they are being released from storage sites following nerve stimulation and that they are turning over, and data concerning (3) the presence in the brain for a number of them of specific binding sites with a regional distribution, (4) their effects on behavioral, neuroendocrine and autonomic processes, (5) their electrophysiological effects and (6) their effects on other brain neuroregulator systems. As was indicated before, this review will only deal with the biochemical effects of ACTH, α -MSH, β -LPH and their derivatives, on other, and specifically the 'classical' neuroregulators in the brain. Since data from the other disciplines will be the subject of parallel reviews in this series, these will only be mentioned for the sake of clarity in selected cases. Hence, results of neuroanatomical studies showing a close association between peptidergic systems and, e.g., catecholamine-containing systems, though indicative for possible interactions between the two, will not be discussed. Neither will results be included of either studies in which the effects were measured of microinjections of peptides of the ACTH-, MSH- and β -LPH-series in brain regions rich in cell bodies or terminals of a particular transmitter on behavioral, neuroendocrine or autonomic parameters, or of studies concerning the effects of their microiontophoretic application in such regions on electrophysiological parameters.

Most of the neurochemical studies that have been undertaken so far were carried out in an attempt to relate effects of peptides on neurotransmitter metabolism with their effects on behavior or, in some cases, neuroendocrine processes. As has been mentioned before, for ACTH and α -MSH and their analogs behavioral effects were mainly those on the acquisition and maintenance of avoidance behavior. The opioid peptides have mainly been studied in relation to their analgesic and cataleptic effects, although they also have been shown to affect other processes like, for instance, avoidance behavior (De Wied *et al.*, 1978a, b), grooming behavior (Gispén *et al.*, 1976), the release of pituitary hormones (Ferland *et al.*, 1977; Du Pont *et al.*, 1979; Holaday and Loh, 1979; Meites *et al.*, 1979; Taché *et al.*, 1979; Van Vugt *et al.*, 1979), blood pressure regulation (Laubie *et al.*, 1977; Bolme *et al.*, 1978; Lemaire *et al.*, 1978; Simon *et al.*, 1978; Bellet *et al.*, 1979; Fuxe *et al.*, 1979b), thermoregulation (Bloom *et al.*, 1976; Bläsig *et al.*, 1978; Holaday *et al.*, 1978; Clark, 1979; Lin *et al.*, 1979; Scoto *et al.*, 1979) and food and water intake (Grandison and Guidotti 1977; Margules *et al.*, 1978; De Caro *et al.*, 1979; King *et al.*, 1979). The doses needed in order to induce analgesia are rather high when compared to those which affect some of the other processes mentioned above (De Wied *et al.*, 1978a, b). It has been suggested that the action of LPH-analogs on some of these latter

processes are mediated by receptor types which have characteristics different from those involved in the analgesic effects (De Wied *et al.*, 1978a, b; Bohus 1980; Van Nispen and Greven 1980). It is therefore that, in many cases, the doses of the opioid peptides used in the various biochemical studies are mentioned.

In many of the studies reviewed here the effects of the opioid peptides on brain neurotransmitters are compared with those of morphine and other opiates and, not surprisingly, found to be similar. In this review the effects of the opiates will not be discussed. Reviews concerning the interaction of opiates with cholinergic (Domino 1979), catecholaminergic (Iwamoto and Way 1979) and serotonergic and GABAergic (Brase 1979) systems in the brain have been published recently.

2. ACTH AND RELATED PEPTIDES

2.1. INTERACTION WITH BRAIN CATECHOLAMINES

The first attempts to obtain information concerning a possible involvement of brain catecholamines in the effects of ACTH and ACTH analogs on animal behavior were made some ten years ago. Both Weiss *et al.* (1970) and Hökfelt and Fuxe (1972), the former on basis of changes in the rate of disappearance of noradrenaline from the brain following synthesis inhibition with α -methyl-*p*-tyrosine methylester (α -MPT), the latter on basis of changes in formaldehyde-induced catecholamine fluorescence following α -MPT, suggested the existence of a correlation between the rate of extinction of conditioned avoidance behavior and central noradrenaline turnover. In other words, these authors suggested that an increased rate of extinction of a conditioned avoidance response was dependent on a decrease of the turnover of noradrenaline in the brain, whereas a decreased rate of extinction was related to an enhanced central noradrenaline turnover. Although this was an interesting working hypothesis, it can now be regarded to have been an oversimplification which was primarily based on the effects of hypophysectomy and adrenalectomy. Hypophysectomy, which decreases circulating ACTH to zero, and is associated with an increased rate of extinction of avoidance behavior (De Wied, 1966, 1969; Bohus 1980), causes a decreased brain noradrenaline turnover (Weiss *et al.*, 1970; Fuxe *et al.*, 1970; Versteeg *et al.*, 1972; Hökfelt and Fuxe 1972; Van Loon 1973), whereas adrenalectomy, which increases circulating ACTH and is associated with a decreased rate of extinction of avoidance behavior (De Wied 1966, 1969; Bohus 1980), is accompanied by an increased noradrenaline turnover in the brain (Javoy *et al.*, 1968; Weiss *et al.*, 1970; Hökfelt and Fuxe (1972). According to the above mentioned suggestion, treatment with ACTH or ACTH fragments, which had been found to inhibit the extinction of avoidance behavior of intact rats (De Wied 1966, 1969; De Wied *et al.*, 1972), should cause an acceleration of the turnover of noradrenaline in the brain. In fact, Hökfelt and Fuxe (1972) reported that ACTH indeed had been found to increase noradrenaline turnover in the hypothalamus, cortex and other brain regions of intact rats. However, whereas treatment of hypophysectomized rats with ACTH or ACTH fragments leads to an improvement of the capacity of these rats to acquire and maintain a conditioned avoidance response (De Wied, 1966, 1969; De Wied *et al.*, 1972), such a treatment was reported to have no effect on central noradrenaline turnover of these rats (Fuxe *et al.*, 1970), albeit that the dose used by these authors, 2.5–10 mU ACTH_{1–24} per rat per day for three days, might have been too low.

Subsequently, it was found by Versteeg (1973) that ACTH_{4–10}, which, like ACTH, inhibits the extinction of avoidance behavior (De Wied 1969; De Wied *et al.*, 1972), increases noradrenaline turnover in whole brain and brain stem. (D-Phe⁷)-ACTH_{4–10}, which has an effect on the extinction of avoidance behavior opposite to that of ACTH_{4–10} (De Wied 1969; De Wied *et al.*, 1972), however, had no effect on noradrenaline turnover (Versteeg 1973). Leonard (1974) found the effects of ACTH_{4–10} and (D-Phe⁷)-ACTH_{4–10} following chronic systemic administration to be qualitatively similar, in that the two peptides caused a slight but significant increase in whole brain

noradrenaline turnover. The results of these two studies were certainly not in favour of the existence of a simple relationship between brain noradrenaline turnover and the performance of rats in tests of the acquisition and maintenance of a conditioned avoidance response.

In experiments in which the capacity of the brain to convert ^3H -tyrosine into ^3H -catecholamines was measured following semi-chronic peptide treatment, (two doses, 72 and 24 hr before the measurement respectively), ACTH_{4-10} was found to increase whole brain and brain stem ^3H -catecholamine synthesis (Versteeg and Wurtman, 1975). However, this effect could not be found following this same treatment either of rats that had been hypophysectomized 18 days before the first treatment, or on the day of the first treatment, or of rats that had been subjected to bilateral adrenalectomy 3 days before the first treatment or on the day of the first treatment (Versteeg and Wurtman, 1975). It should be noted that in the experiments with intact rats Versteeg and Wurtman (1975) measured the accumulation of total ^3H -catecholamines; it is thus unclear, whether the effect is due to an increased synthesis of noradrenaline, of dopamine, or of both catecholamines. Using a similar treatment schedule, and comparable biochemical methods, Dunn and associates observed that in mice ACTH_{1-24} , ACTH_{4-10} and $(\text{D-Phe}^7)\text{-ACTH}_{4-10}$ caused an increase in the conversion of ^3H -tyrosine into ^3H -dopamine in the brain, but not into ^3H -noradrenaline (Dunn *et al.*, 1976; Iuvone *et al.*, 1978). Interestingly, Iuvone *et al.* (1978) also found that ACTH_{4-10} was unable to affect the conversion of ^3H -tyrosine into ^3H -dopamine in the brains of rats that had been adrenalectomized 24 hr before the first peptide administration.

Results of studies with $\alpha\text{-MSH}$ ($(\text{N-acetylSer}^1)\text{-ACTH}_{1-13}$), which contains the behaviorally active sequence of ACTH_{4-10} , on $\alpha\text{-MPT}$ -induced catecholamine disappearance in the brain of intact and hypophysectomized rats, led Kostrzewa *et al.* (1975) to conclude that a correlation between effects on behavioral parameters and effects on noradrenaline turnover in the brain did not appear to be very likely for $\alpha\text{-MSH}$. In agreement with the findings of Kostrzewa *et al.* (1975), Dunn *et al.* (1976) and Iuvone *et al.* (1978) observed no effects of either α - or β -MSH on the conversion of ^3H -tyrosine into ^3H -catecholamines in mouse brain. Taken together, it seems to be unresolved whether the effects of ACTH-like peptides, which all have the ACTH_{4-10} sequence in common on brain catecholamines is a direct one; it seems likely that factors of an as yet unidentified nature are involved.

Also the results of studies in which the effect of treatment with ACTH fragments was measured on regional tyrosine hydroxylase (TH) activity in the brain, are conflicting. Initially, Dunn and associates reported that in mice treated for 3 days with ACTH_{4-10} , striatal TH activity was 50–60% higher than that of vehicle-treated animals (Dunn *et al.*, 1976). In a more extensive study however, Dunn *et al.* (1978) were unable to find any significant effect of this peptide, or of ORG2766 , a protected ACTH_{4-9} derivative which has been shown to be very potent in behavioral tests (Van Nispen and Greven 1980; Bohus, 1980), on TH activity in mouse striatum or in any of the other brain parts studied. Van Loon *et al.* (1978b) reported that in rats hypophysectomized 14 days before decapitation, TH activities of the hypothalamus, pons-medulla and cerebellum were increased by approximately 20% over that of weight-matched controls, while TH activity of the striatum was unaffected. Chronic treatment of hypophysectomized rats with a low dose of ACTH_{1-24} (0.5 $\mu\text{g}/100$ g body weight, s.c. per day for 12 days) largely prevented the hypophysectomy-induced rise in TH activity of the hypothalamus, partly prevented the increase in the pons-medulla and had no effect on TH activity of the cerebellum and striatum (Van Loon *et al.*, 1978b). Similarly, it was found that ACTH treatment of hypophysectomized rats counteracted the hypophysectomy-induced decrease in dopamine- β -hydroxylase activity of the hypothalamus and pons-medulla, but not of the cerebellum (Van Loon *et al.*, 1978b). In contrast to these findings with hypophysectomized rats, however, Van Loon *et al.* (1978b) observed that treatment of intact rats with ACTH_{1-24} failed to produce any changes in regional TH activity in the brain. Again, therefore, it cannot be said that these data on effects and lack of effects of ACTH-related

peptides on the enzymes involved in brain catecholamine synthesis form evidence in favour of a correlation between behavioral effects and effects on brain catecholamines of these peptides.

Whereas the results of the above described studies do not seem to support that the effect of ACTH analogs on brain catecholamine systems is a direct one, the results of Endröczi and associates seem to indicate the opposite. Intracerebroventricular (i.c.v.) administration of doses of ACTH₁₋₂₄ and ACTH₄₋₁₀ of 0.1 up till 5.0 µg caused an increase in the rate of disappearance of i.c.v. administered ³H-noradrenaline from the hypothalamus, hippocampus and cortex of adrenalectomized rats (Endröczi *et al.*, 1975; Endröczi 1976). Implantation of ACTH₄₋₁₀ in the region of the locus coeruleus, but not in the septal region or in the medial forebrain bundle at the level of the posterior hypothalamus, also caused an increase in the disappearance of label from hippocampus, hypothalamus and cortex (Endröczi, 1977). These results implicate an effect of bio-active ACTH analogs at the level of the cell bodies of the dorsal noradrenergic bundle, which might be related to the effects of these peptides on behavior (Endröczi, 1977). It should be realized, however, that the degradation of i.c.v. injected ³H-noradrenaline is different from that of endogenous noradrenaline and also that ³H-noradrenaline can be taken up by other neurons than noradrenaline neurons as well.

Due to the technique employed, the work of Lichtensteiger and associates has a special and somewhat isolated position. In a pilot study Lichtensteiger *et al.* (1976) established that a close relationship exists between the mean fluorescence intensity of a given group of dopamine neurons, as measured by a microfluorometric procedure based on the formaldehyde-induced fluorescence method of Falck and Hillarp, and their firing rate; a rise in mean fluorescence intensity being accompanied by an increase in mean firing rate. Using this complicated but very sensitive technique Lichtensteiger and associates were able to study the effects of treatment with peptides on the functional state of groups of catecholamine neurons. Lichtensteiger and Lienhart (1977) observed that 30 min following the administration of α-MSH in doses ranging from 10 to 100 µg/kg, i.p. dopamine containing neurons in the arcuate nucleus and the substantia nigra responded with an increased firing rate. Comparable doses of ACTH₁₋₂₄, on the other hand, were without effect (Lichtensteiger and Lienhart 1977). In a more recent study, Lichtensteiger *et al.* (1979) and Lichtensteiger and Monnet (1979) reported that arcuate nucleus dopamine neurons were activated following treatment of rats with α-MSH and also after administration of its C-terminal tripeptide α-MSH₁₁₋₁₃, whereas little, if any, effect was seen with ORG2766, which has a high potency in affecting behavior but is devoid of MSH activity. On basis of these findings it was concluded (Lichtensteiger *et al.*, 1979; Lichtensteiger and Monnet 1979) that the response of the arcuate nucleus dopamine neurons was related to activation of a neuroendocrine feedback loop involved in the regulation of MSH secretion. On the activity of dopamine neurons in the substantia nigra, however, ORG2766 was most potent in affecting dopamine fluorescence intensities (Lichtensteiger *et al.*, 1979; Lichtensteiger and Monnet 1979). Obviously, several discrepancies need to be resolved, one of them being that ORG2766, particularly in the higher dose range (5.0 µg/kg), did cause a decrease rather than an increase in mean fluorescence intensity (Lichtensteiger *et al.*, 1979), while ACTH₁₋₂₄ had no effect and α-MSH increased dopamine fluorescence intensities (Lichtensteiger and Lienhart 1977). In any case, again there seems not to be a relationship with the behavioral effects of these three peptides as observed by De Wied and associates (De Wied 1966, 1969; De Wied *et al.*, 1972; De Kloet and De Wied 1980; Bohus 1980).

In addition to the above mentioned data, which concern the results of studies in which the effects of various ACTH analogs were measured on dynamic aspects of brain catecholamine metabolism, a number of results has to be mentioned concerning the influence of ACTH and ACTH fragments on the concentration of catecholamines in whole brain, brain parts or microdissected brain nuclei. In several cases these data on catecholamine concentrations were by-products of measurements of the rate of turnover of catecholamines following synthesis inhibition with α-MPT (Versteeg 1973; Leonard 1974; Kostr-

zewa *et al.*, 1975). In those cases that levels of catecholamines were measured in whole brain (Versteeg 1973; Leonard 1974; Iuvone *et al.*, 1978) or in brain parts (Versteeg 1973; Leonard 1974; Ramaekers *et al.*, 1978; Kostrzewa *et al.*, 1975) following chronic or semi-chronic treatment with ACTH analogs, not or only marginal effects were found. Telegdy and Kovács (1979a, b), however, observed an increased dopamine concentration in striatum and mesencephalon, but not in hypothalamus, septum and hippocampus, 30 min after the administration of 20 μg ACTH per rat, i.p. while this treatment caused a decrease in the concentration of noradrenaline in septum and hippocampus, but not in the other three brain parts. Using a sensitive radio-enzymatic catecholamine assay, Fekete *et al.* (1975, 1978) measured the effects of acute ACTH administration on catecholamine levels of brain nuclei dissected as described by Palkovits (1973). In their first paper, Fekete *et al.* (1975) reported that 30 min after 30 μg ACTH the dopamine concentration of the median eminence and the noradrenaline concentration of the supraoptic nucleus were increased, whereas the dopamine concentration of the locus coeruleus was decreased; 3 hr after ACTH administration the noradrenaline level was elevated in the locus coeruleus and decreased in the supraoptic nucleus, while the dopamine concentration of the arcuate nucleus was decreased. In a subsequent paper (Fekete *et al.*, 1978) no changes were reported to occur in the dopamine concentration of any of the 12 brain regions 3 hr after ACTH administration. The noradrenaline concentration of the locus coeruleus was increased at that time; that of the arcuate nucleus and the ventromedial nucleus decreased (Fekete *et al.*, 1978). Apart from the apparent lack of consistency, another problem with these results is their interpretation. Absence of differences in catecholamine concentrations following peptide and vehicle treatment does not necessarily include the absence of differences in turnover or synthesis, as is indicated by the results of Versteeg (1973), Leonard (1974), Kostrzewa *et al.* (1975) and Iuvone *et al.* (1978). On the other hand, if changes in catecholamine concentrations are found, it is not possible, without additional data, to ascribe the change in concentration to either an increased or a decreased neuronal activity.

2.2. INTERACTION WITH BRAIN ACETYLCHOLINE

Following i.c.v. administration, ACTH₁₋₂₄ and α -MSH, in a dose of 10 μg per rat, have been found to induce an increase in hippocampal acetylcholine turnover (Wood *et al.*, 1978). No effects were evident on acetylcholine turnover in cortex, striatum and diencephalon, while on the turnover of brain stem acetylcholine only α -MSH had effects (Wood *et al.*, 1978). In these experiments the turnover of acetylcholine was measured by infusing i.v. phosphoryl-(²H₉) choline in a rate of 15 μmol per min for 9 min starting 21 min after the i.c.v. administration of the peptide, and then, after decapitation of the rat, calculating the ²H enrichment of choline and acetylcholine as measured by means of mass fragmentography (Zsilla *et al.*, 1977). ACTH₁₋₂₄, α -MSH and a number of analogs of these peptides have been found to induce a stretching and yawning syndrome which is preceded by excessive grooming (for references see Wood *et al.*, 1978; Gispen and Isaacson 1980). On basis of the fact that this stretching and yawning syndrome can be prevented by centrally acting anticholinergic drugs (Ferrari *et al.*, 1963), Wood *et al.* (1978) suggest that the effect of ACTH₁₋₂₄ and α -MSH on stretching and yawning which they observe might be related to the effects of these peptides on hippocampal acetylcholine turnover. Interestingly, ORG2766, which, as was mentioned before, is very potent in facilitating the retention of conditional avoidance behavior, fails both to induce stretching and yawning and to affect hippocampal acetylcholine turnover (Wood *et al.*, 1978). It should be noted, however, that brain dopamine and in particular the nigrostriatal and mesolimbic dopamine systems have been implicated in the excessive grooming elicited by i.c.v. administration of ACTH₁₋₂₄, α -MSH and a number of analogs, e.g. ACTH₁₋₁₆ and (D-Phe⁷)-ACTH₄₋₁₀, but not ACTH₄₋₁₀ (Wiegant *et al.*, 1977; Cools *et al.*, 1978).

In further experiments Wood *et al.* (1979) showed that 2 to 3 weeks after surgical

transection of the projections from the cingulum or the entorhinal cortex to the hippocampus ACTH₁₋₂₄ and α -MSH still can enhance hippocampal acetylcholine turnover. Neither lesioning of the arcuate nucleus, which contains the cell bodies of the ACTH/MSH system, nor acute transection of the fimbria interfered with the effects of the peptides on hippocampal acetylcholine turnover (Wood *et al.*, 1979). On the basis of these findings and on the finding that intraseptal injection of ACTH₁₋₂₄ or α -MSH failed to affect hippocampal acetylcholine turnover Wood *et al.* (1979) suggest that these peptides exert their stimulating effect on the turnover of acetylcholine in the hippocampus by activating receptors located in the hippocampus.

2.3. INTERACTION WITH BRAIN SEROTONIN

While many papers have been published reporting effects of ACTH analogs on brain catecholamines, few data are available on effects of this group of peptides on brain serotonin. Leonard (1974) found that chronic treatment with ACTH₄₋₁₀ or (D-Phe⁷)-ACTH₄₋₁₀ (10 μ g for 13 days) resulted in a decrease in the concentration and turnover of serotonin in the rat brain. In a more recent paper Leonard *et al.* (1976) reported an increased conversion of ³H-tryptophan into ³H-serotonin in rat cortex, but not in other brain regions, following chronic treatment with α -MSH. Spirtes *et al.* (1975), on the other hand, found no effect of α -MSH on serotonin concentration and serotonin turnover in the brains of intact rats, while serotonin accumulation after pargyline was decreased in the cortex of hypophysectomized rats following treatment with α -MSH. Acute effects of ACTH (20 μ g per rat, 30 min prior to decapitation) were observed on the serotonin concentration of the hypothalamus, mesencephalon and hippocampus by Telegdy and Kovács (1979a; 1979b). Again, like the case with the catecholamines, it can be concluded that there is no convincing evidence among the available data for a correlation between effects of ACTH-like peptides on serotonin metabolism in the brain and the effects of these peptides on behavior. The results of a study by Ramaekers *et al.* (1978) seem to be the only exception. These authors, comparing the content of noradrenaline, dopamine and serotonin in a number of brain regions of the rat in relation to passive avoidance training and treatment with ACTH₄₋₁₀ and (D-Phe⁷)-ACTH₄₋₁₀, found that changes in hippocampal serotonin levels, but not in other brain parts or in catecholamine levels in any of the brain parts studied, were correlated to the changes in passive avoidance behavior after the administration of the analogs (Ramaekers *et al.*, 1978). An obvious criticism of this work however, is that, since catecholaminergic activity can be subject to considerable changes in the absence of changes in catecholamine levels, the measurement of catecholamine concentrations only can easily lead to false negative conclusions.

3. ENKEPHALINS

3.1. INTERACTION WITH BRAIN CATECHOLAMINES

A relatively large number of data is available concerning effects of Met-enkephalin and its more stable synthetic analogs, administered in doses which are known to cause analgesia and catalepsy, on striatal dopamine in the rat. Effects have been described on (1) homovanillic acid (HVA) and 3,4-dihydroxyphenyl-acetic acid (DOPAC) levels, (2) the accumulation of these two dopamine metabolites following probenecid administration, (3) the rate of disappearance of dopamine following inhibition of its synthesis, (4) the conversion of ³H-tyrosine into ³H-dopamine and (5) the accumulation of 3,4-dihydroxyphenylalanine (DOPA) following inhibition of DOPA-decarboxylase with NSD 1015. In addition to this, effects have been reported on K⁺-induced release of ³H-dopamine from striatal slices *in vitro*. Though the results in many instances are unequivocal, the majority of these data support the notion that the enkephalins increase dopamine metabolism in this brain part. In agreement with the fact that analogs like D-Ala²-Met-enkephalinamide are more resistant to enzymatic degradation than the naturally occurring enkepha-

lins, effective doses of these stable peptides are considerably lower than those of e.g. Met-enkephalin.

Loh *et al.* (1976) reported that, whereas β -endorphin had an inhibitory influence on ^3H -dopamine release from striatal slices induced by potassium ions in a concentration of 53 mM, under these conditions Met-enkephalin in concentrations as high as 10^{-4} M was ineffective in doing so. Using similar experimental conditions however, Subramanian *et al.* (1977), found that Met-enkephalin in concentrations of 10^{-6} and 10^{-5} M significantly diminished the K^+ -induced (40 mM) release of ^3H -dopamine from striatal slices *in vitro*, an effect which could be blocked by naloxone. The reason for this discrepancy is not known, although it has been suggested that the K^+ -concentration used by Loh *et al.* (1976) might be physiologically high (cf. Arbilla and Langer, 1977).

Algeri *et al.* (1977) found that 45 min after the i.c.v. administration of D-Ala²-Met-enkephalinamide in doses of 25 or 50 μg per rat striatal HVA concentrations were increased significantly; following 10 μg per rat striatal HVA levels were not significantly elevated, neither were DOPAC levels after any of the doses. In contrast, no differences were observed in striatal HVA content either 15 min after the i.c.v. administration of 100 μg Met-enkephalin or 30 min after two doses of 100 μg of this peptide given with a 15 min interval (Algeri *et al.*, 1977). Similarly, Berney and Hornykiewicz (1977) found no effect on striatal HVA concentrations after the i.c.v. administration of 100 μg Met-enkephalin. Although this difference in effectiveness to elevate striatal HVA levels between Met-enkephalin and the D-Ala² analog might be due, as was suggested by Algeri *et al.* (1977; Calderini *et al.*, 1978), to the difference in susceptibility of the two peptides to enzymatic degradation, it obviously cannot be excluded that other factors have to be taken into account. Using a similar experimental design, Biggio *et al.* (1978a) found that doses of D-Ala²-Met-enkephalinamide as low as 1.5 or 3.0 μg per rat i.c.v. caused a significant increase in the levels of both HVA and DOPAC in the caudate nucleus, while doses of Met-enkephalin of 50 or 100 μg per rat resulted in elevations of the content of the dopamine metabolites of an order of magnitude comparable to that after 1.5–3.0 μg of the D-Ala²-analog. It was also found that naloxone, given i.p. at the same time as the peptides were administered i.c.v., completely antagonized the effects of both Met-enkephalin and D-Ala²-Met-enkephalinamide on caudate HVA and DOPAC concentrations (Biggio *et al.*, 1978a). Injections of D-Ala²-Met-enkephalinamide directly into the caudatus also caused an increase of the DOPAC content in this nucleus, demonstrating that the caudate is the site of action of the peptide (Biggio *et al.*, 1978a). The D-Ala² analog, given in doses of 3.0 or 6.0 μg i.c.v. caused an increase in the accumulation of DOPA in the caudate nucleus following DOPA-decarboxylase inhibition with NSD 1015 (Biggio *et al.*, 1978a), an effect which has also been observed in the striatum with very high doses of Met- and Leu-enkephalin (Magnusson *et al.*, 1979) and with the enkephalin analogs D-Ala²-Met-enkephalinamide (Magnusson *et al.*, 1979; Garcia-Sevilla *et al.*, 1980) and D-Ala²-MePhe⁴-Met(O)⁵-ol-enkephalin (FK 33-824) (Garcia-Sevilla *et al.*, 1980) at a much lower dose level. On basis of their data, Biggio *et al.* (1978a) concluded that the enkephalins cause an elevation of dopamine synthesis by activating opioid receptors in the caudate nucleus. Since the prior destruction of caudate neurons postsynaptic to the nigrostriatal dopamine terminals did not prevent the effect of D-Ala²-Met-enkephalinamide either on DOPAC content or on DOPA accumulation following NSD 1015, it was also concluded that the peptide exerts its effect on caudate dopamine synthesis via an action on opioid receptors located on the dopamine terminals rather than on postsynaptic structures (Biggio *et al.*, 1978a, b).

The suggestion that the opioid receptors exerting an influence on caudate nucleus dopamine metabolism are located on the dopamine terminals of nigrostriatal neurons is further supported by the results of studies in which the binding of different radiolabeled ligands for opioid binding sites was measured to striatal membranes obtained from rats that had been subjected to various chemical or electrical lesions. Thus, it was shown that degeneration of caudate nucleus terminal, either by i.c.v. 6-hydroxydopamine (6-OHDA) administration or by electrocoagulation of the pars compacta of the substantia nigra,

resulted in approximately 30% decrease in ^3H -Leu-enkephalin and/or ^3H -naloxone binding in the striatum (Pollard *et al.*, 1977). This, combined with the finding that not more than a 30–40% decrease in ^3H -Leu-enkephalin binding was observed after transection of the nigrostriatal dopamine pathway at the level of the hypothalamus (Pollard *et al.*, 1978), indicates that not more than one-third of the opioid receptors in the striatum is located on dopamine terminals, while the remaining two-thirds are supposed to be located on neurons intrinsic to or projecting from this structure (Pollard *et al.*, 1977, 1978; Schwartz *et al.*, 1978, 1979). Similar results were reported by Carenzi *et al.* (1978) and Trabucchi *et al.* (1979), using ^3H -D-Ala²-Met-enkephalinamide binding following various lesions.

Data concerning effects of enkephalin analogs on striatal dopamine turnover and on the striatal conversion of ^3H -tyrosine into ^3H -dopamine, although in many cases fitting with the concept of an enhanced dopamine metabolism, are sometimes conflicting. Using the *in vivo* capacity of the striatum to convert ^3H -tyrosine into ^3H -dopamine, Calderini *et al.* (1978) found that, whereas D-Ala²-Met-enkephalinamide, injected i.c.v. in a dose of 10 μg per rat, caused a significant increase in striatal dopamine synthesis, Met-enkephalin, in a dose of 200 μg per rat administered via the same route, caused a significant decrease. Similarly, DOPAC and tritiated DOPAC concentrations were decreased after injection of Met-enkephalin and increased when the D-Ala² analog had been administered; the concentration of ^3H -3-methoxytyramine, however, was decreased after Met-enkephalin, but not affected after D-Ala²-Met-enkephalinamide administration (Calderini *et al.*, 1978). This latter observation, according to Calderini *et al.* (1978), is in agreement with the assumption that D-Ala²-Met-enkephalinamide acts mainly on intraterminal dopamine synthesis, while dopamine release is decreased (cf. Subramanian *et al.*, 1977).

Based on the fact that following Met-enkephalin administration the level of cold tyrosine and tritiated tyrosine is markedly elevated in the striatum, Calderini *et al.* (1978) postulated that the effect of Met-enkephalin on striatal dopamine synthesis might be an artifact caused by the rapid release of the tyrosine residue from the injected peptide by intracerebral enzymatic degradation (cf. Hambrook *et al.*, 1976; Meek *et al.*, 1977; see also De Kloet *et al.*, 1980). Recently, Garcia-Sevilla *et al.* (1980) reported that both Met-enkephalin and Leu-enkephalin, but not D-Ala²-Met-enkephalinamide and D-Ala²-MePhe⁴-Met(O)⁵-ol-enkephalin, caused a marked increase of the tyrosine concentration in limbic regions, hemispheres, diencephalon and lower brain stem, while the effect in the striatum was much less pronounced. Anyhow, since Met-enkephalin, but not its D-Ala² analog, was found to cause a decrease in the conversion of ^3H -tyrosine into ^3H -noradrenaline also in the cortex (Calderini *et al.*, 1978), the explanation of these authors seems to be a tenable one. Nevertheless, the final proof, e.g. by measuring the effect of an amount of tyrosine equivalent to that set free from 200 μg Met-enkephalin on ^3H -catecholamine synthesis, was not given.

Striatal dopamine turnover, as measured from the rate of dopamine disappearance following inhibition of tyrosine hydroxylase with α -MPT, was found to be increased after the i.c.v. administration of 25 μg D-Ala²-Met-enkephalinamide (Calderini *et al.*, 1978) and D-Ala²-D-Leu⁵-enkephalin (Deyo *et al.*, 1979). Schwarcz *et al.* (1979), on the other hand, reported that Met-enkephalin, given as an i.c.v. infusion of approximately 50 μg over a period of one hr in a total volume of 60 μl , did not affect the α -MPT-induced disappearance of dopamine fluorescence from dopamine terminals in the caudate nucleus. Using the considerably lower dose of 100 ng Met-enkephalin per rat, i.c.v. Versteeg *et al.* (1978) found no effect of this peptide on the α -MPT-induced disappearance of dopamine from either the caudate nucleus or the nucleus accumbens, though there were effects in other brain regions. In a recent paper Garcia-Sevilla *et al.* (1980) reported that after inhibition of DOPA-decarboxylase with benserazide the disappearance of dopamine was enhanced following the i.c.v. administration of 500 μg of either Met- or Leu-enkephalin. Similar results were obtained with D-Ala²-Met-enkephalin (50 μg) and D-Ala²-MePhe⁴-Met(O)⁵-ol-enkephalin (1 μg) (Garcia-Sevilla *et al.*, 1980). Differences in dose

level seem to be the most plausible explanation for the observed differences in effectiveness of Met-enkephalin in eliciting changes in dopamine disappearance following synthesis inhibition.

The majority of the above described data can be considered to be compatible with the suggestion of Pollard *et al.* (1978) and Schwartz *et al.* (1978) that the primary effect resulting from the activation by enkephalins of opioid receptors localized presynaptically on dopamine terminals in the striatum is a decrease in dopamine release, which accounts for the various symptoms of decreased dopaminergic transmission observed immediately after their administration; secondary to this effect the interaction of the enkephalins causes an increased dopamine synthesis, due to a diminished feedback inhibition of presynaptic dopamine receptors, as can be demonstrated by increases in various parameters estimating dopamine synthesis. However, it is likely that additional mechanisms are involved in the interaction of enkephalins with striatal dopamine activity, as is indicated by Biggio *et al.* (1978a) and Algeri *et al.* (1978a; 1979).

Whereas an abundance of data is available concerning the effects of enkephalin analogs on striatal dopamine metabolism, less is known concerning effects on catecholamines in other brain regions. The first evidence for an interaction of enkephalins with brain catecholamines dates from 1976, when Taube *et al.* (1976) reported that Met-enkephalin, in concentrations of 10^{-5} down to 10^{-7} M, diminished the release of label evoked by electrical field stimulation or by 20 mM K^+ from slices of rat occipital cortex preloaded with 3H -noradrenaline. This effect was antagonized by naloxone, but not by the α -blocker phentolamine (Taube *et al.*, 1976). It was concluded that Met-enkephalin reduces transmitter release from cortical noradrenergic terminals, and that one function of endogenous opioid peptides might be the presynaptic inhibition of central noradrenergic neurotransmission (Taube *et al.*, 1976). Recently, these findings were corroborated by Göthert *et al.* (1979), who found that 3H -noradrenaline release promoted by 30 mM K^+ from cortical and also from hypothalamic slices was decreased by Met-enkephalin at a concentration of 10^{-6} M.

Studies on effects of enkephalin analogs on *in vivo* catecholamine metabolism, as was the case with those on effects on striatal dopamine, have been carried out using a variety of methods, injection schedules, doses and dissection procedures. D-Ala²-Met-enkephalinamide, 25 μ g per rat administered i.c.v. increased the accumulation of DOPAC and HVA following probenecid and the conversion of 3H -tyrosine into 3H -dopamine in a brain region consisting of the nucleus accumbens, the tuberculum olfactorium and the amygdala (Calderini *et al.*, 1978). Schwarcz *et al.* (1979) found that an i.c.v. infusion of Met-enkephalin causes an increase in the α -MPT-induced disappearance of dopamine from the tuberculum olfactorium and the anterior part of the nucleus accumbens. D-Ala²-Met-enkephalinamide administered i.c.v. in a dose of 25 μ g, according to Calderini *et al.* (1978) and Algeri *et al.* (1978a), does not affect either the rate of disappearance of noradrenaline after synthesis inhibition or the conversion of 3H -tyrosine into 3H -noradrenaline in the cortex. Higher doses, however, appear to be effective, as was shown by Garcia-Sevilla *et al.* (1980). These authors found that the enkephalin analogs D-Ala²-Met-enkephalin and D-Ala²-MePhe⁴-Met(O)⁵-ol-enkephalin dose dependently (4–256 μ g for the former peptide; 0.003–1 μ g for the latter) increased the accumulation of DOPA following NSD 1015 in the hemispheres and also in limbic regions, diencephalon and lower brain stem. Large doses of Met-enkephalin and Leu-enkephalin (500 μ g per rat, i.c.v.) were needed in order to obtain a significant effect on this parameter (Garcia-Sevilla *et al.*, 1980).

The α -MPT-induced disappearance of dopamine from the cortex and from the median eminence was found to be decreased by D-Ala²-D-Leu⁵-enkephalin by Deyo *et al.* (1979). This latter observation is in accordance with results of a microfluorometric study by Ferland *et al.* (1977) of the α -MPT-induced disappearance of formaldehyde-induced catecholamine fluorescence from substructures of the median eminence. These authors observed, using an administration schedule identical to that described above for the study by Schwarcz *et al.* (1979), a reduction in the disappearance of catecholamine

fluorescence from the medial pallisade zone of the median eminence, which contains dopamine and noradrenaline terminals, and from the lateral pallisade zone, which contains mainly dopamine terminals. It has been suggested that this reduction of dopamine turnover in the median eminence is related to the effect of the opioid peptides on prolactin release from the pituitary (see Ferland *et al.*, 1977; Deyo *et al.*, 1979; also for references), which effect, as such, falls outside the scope of this review.

Using a much lower dose of Met-enkephalin (100 ng per rat) Versteeg *et al.* (1978) found the α -MPT-induced disappearance of noradrenaline to be enhanced in the medial preoptic nucleus and the central amygdaloid nucleus, and to be decreased in the ventral central gray and the A2 region of the nucleus tractus solitarii, while the disappearance of dopamine was increased in the arcuate nucleus and the dorsal central gray. Taken together, it is evident that, as far as effects of enkephalins on catecholamine metabolism in brain regions other than the striatum are concerned, our knowledge is only fragmentary. Also, in many cases the significance of the observed changes is, as yet, obscure.

3.2. INTERACTION WITH BRAIN ACETYLCHOLINE

Met-enkephalin, in concentrations of 10^{-6} and 10^{-5} M, has been found to reduce the K^+ -induced release *in vitro* of radioactivity from hippocampal slices preloaded with ^3H -acetylcholine, by incubating the slices with labeled choline (Subramanian *et al.* (1977). This effect, again, was prevented by naloxone (Subramanian *et al.*, 1977). Using an *in vivo* cup technique, Jhamandas *et al.* (1977) obtained similar results for the spontaneous release of cortical acetylcholine. Both Met-enkephalin and Leu-enkephalin, given *i.c.v.* in a dose of 10 μg , were found to reduce acetylcholine release significantly, an effect which was counteracted by the subsequent administration of naloxone and prevented by the prior administration of this drug (Jhamandas *et al.*, 1977).

The situation in the striatum seems to be more complicated, as is indicated by the results of Vizi *et al.* (1977) and Harsing *et al.* (1978). Met-enkephalin methylester and D-Met²-Pro⁵-enkephalinamide (Harsing *et al.*, 1978) and D-Ala²-Pro⁵-enkephalinamide (Vizi *et al.*, 1977; Harsing *et al.*, 1978) all caused an increase in ouabain-induced acetylcholine release from rat striatal slices. The effect of these peptides was prevented by naloxone (Vizi *et al.*, 1977). However, enkephalin analogs inhibited, rather than increased, the release of acetylcholine from striatal slices of rats which had been pretreated with *i.c.v.* 6-OHDA in doses that cause a destruction of the nigro-striatal dopamine system (Vizi *et al.*, 1977). It was suggested that the effect of enkephalin analogs on acetylcholine release from striatal tissue from intact rats should be ascribed to their decreasing influence on dopamine release from neurons that have an inhibitory control on striatal cholinergic interneurons (Vizi *et al.*, 1977). That enkephalins reduce acetylcholine release from striatal slices of 6-OHDA-pretreated rats indicates that activation of opioid receptors located on the striatal cholinergic neurons might be involved in modulating acetylcholine release, according to Vizi *et al.* (1977) and Harsing *et al.* (1978), in a way similar to that of hippocampal (Subramanian *et al.*, 1977) and cortical (Jhamandas *et al.*, 1977) cholinergic neurons.

3.3. INTERACTION WITH BRAIN SEROTONIN

Whole brain serotonin metabolism appears to be enhanced following high *i.c.v.* doses of Met-enkephalin, as can be seen from the finding by Laska and Fennessy (1978) of an increased 5-HIAA level 3 min after 150–600 μg of the peptide and by Magnusson *et al.* (1979) of an increased accumulation of 5-HTP after NSD 1015 administration following 500 μg Met-enkephalin. The latter authors also found that Leu-enkephalin, in the same dose as that of Met-enkephalin, and D-Ala²-Met-enkephalinamide, in doses of 4 to 250 μg , had effects similar to that of Met-enkephalin (Magnusson *et al.*, 1979). Algeri *et al.* (1978c), in a study on the effects of D-Ala²-Met-enkephalinamide on brain serotonin metabolism, observed that whole brain 5-HIAA levels in whole brain were elevated 45, 90

and 135 min after the i.c.v. administration of this peptide. In addition, Algeri *et al.* (1978c) observed that this dose caused an increase in the accumulation of 5-HIAA in the limbic forebrain following probenecid, but not in the striatum of the rat (see also Algeri *et al.*, 1978b). These effects of the D-Ala² analog were completely counteracted by the prior administration of naloxone, which points at the involvement of opioid receptors in the effects of the peptide on limbic serotonin metabolism (Algeri *et al.*, 1978b, c).

In an extensive follow-up of the study by Magnusson *et al.* (1979), in a recent paper Garcia-Sevilla *et al.* (1980) describe the effects of various enkephalin analogs on regional serotonin metabolism in the rat brain. Met-enkephalin and Leu-enkephalin (500 μg per rat, i.c.v.) both enhanced the formation of 5-HTP in diencephalon and lower brain stem of NSD 1015-pretreated rats. Met-enkephalin caused a small but significant increase in striatum and hemispheres, but not in the limbic forebrain; Leu-enkephalin was ineffective in the latter three brain regions. D-Ala²-Met-enkephalinamide, in a dose of 125 μg per rat, i.c.v. had effects of the same order of magnitude as that of Met-enkephalin in the diencephalon and lower brain stem, but was more effective in enhancing 5-HTP accumulation in limbic forebrain, striatum and hemispheres. A lower dose of the D-Ala²-analog was not effective. D-Ala²-MePhe⁴-Met(O)⁵-ol-enkephalin, 1 μg per rat, caused a moderate increase in 5-HTP formation in all five brain regions. Met- and Leu-enkephalin (500 μg) and also D-Ala²-Met-enkephalinamide (50 μg) caused a decrease in the 5-HT level in the brain of benserazide-pretreated rats. After Met-enkephalin (500 μg) and D-Ala²-MePhe⁴(O)⁵-ol-enkephalinamide (1 μg) brain 5-HIAA levels of benserazide-pretreated rats were increased. Though the above mentioned results suggest that enkephalins are participating in the regulation of the activity of serotonin-containing systems in the brain, the localization of the receptors and the circuitry involved remains to be elucidated.

4. ENDORPHINS

4.1. INTERACTION WITH BRAIN CATECHOLAMINES

Like the case with the enkephalins, the first data concerning biochemical effects of endorphins on brain neurotransmitters were on K⁺-induced release of label from brain slices preloaded with tritiated catecholamines *in vitro*. In 1976 Loh *et al.* reported that β -endorphin (β -LPH₆₁₋₉₁) dose dependently inhibited the K⁺-stimulated release of ³H-dopamine from superfused striatal slices; the effect was obtained with a K⁺ concentration of 53 mM and was antagonized by naloxone. It was in this same paper that Loh *et al.* (1976) reported that Met-enkephalin failed to affect the K⁺-induced release of ³H-dopamine from striatal slices (see Section 3.1.). In experiments in which a K⁺ concentration of 20 mM was used, which can be considered to be a more physiological condition, Arbilla and Langer (1978), however, were unable to find any effect of β -endorphin or morphine on the release of ³H-dopamine from striatal slices. Under the same experimental conditions β -endorphin was found to diminish the K⁺-induced release of ³H-noradrenaline by slices from rat occipital cortex (Arbilla and Langer, 1978).

Data on effects on β -endorphin on *in vivo* parameters of striatal dopamine metabolism, like the conversion of ³H-tyrosine into ³H-dopamine and the α -MPT-induced disappearance of dopamine, also are not without conflict. Izumi *et al.* (1977) found no significant effect of β -endorphin, injected i.c.v. in a dose of 50 μg , on the α -MPT-induced disappearance of dopamine from the striatum. On the other hand, Fuxe *et al.* (1977) and Schwarcz *et al.* (1979), using microspectrofluorometric methods, and Deyo *et al.* (1979) reported that following a dose of β -endorphin of the same order of magnitude, the disappearance of dopamine from the striatum after synthesis inhibition with α -MPT was increased. Van Loon and Kim (1978) also studied the effects of β -endorphin on α -MPT-induced disappearance of dopamine from rat striatum. In their experiments rats received the tyrosine hydroxylase inhibitor 1 hr prior to the intracisternal injection of either saline or 15 μg β -endorphin, and were decapitated 45 or 90 min after the last injection. Interestingly, Van Loon and Kim (1978) observed a significantly lower dopamine concentration in the

striatum of β -endorphin-treated rats than in that of saline-treated rats 45 min after administration of the peptide, whereas no differences were apparent in the striatal dopamine level of both the groups that were killed 90 min after the administration of β -endorphin or saline. This indicated that initially, i.e. in the first 45 min after its injection β -endorphin accelerates, whereas in the second 45 min period it decreases dopamine utilization. It, therefore, seems possible, also taking into account that the peptide supposedly reaches its sites of action more rapidly following i.c.v. than following intracisternal injection, that, when residual dopamine concentrations are measured shortly after i.c.v. administration (Deyo *et al.*, 1979) or after an i.c.v. infusion (Fuxe *et al.*, 1977; Schwarcz *et al.*, 1979), an increased disappearance is evident, while after longer periods no effects (Izumi *et al.*, 1977), or even opposite effects are seen. Versteeg *et al.* (1979), using a much lower dose of 100 ng β -endorphin per rat, i.c.v. found no effect of this peptide on α -MPT-induced dopamine disappearance from either the caudate nucleus or the nucleus accumbens; α -endorphin (β -LPH₆₁₋₇₆), on the other hand, was found to cause a decrease in dopamine utilization in the caudate nucleus. In these experiments peptides or saline were administered 30 min after α -MPT injection and 3 hr prior to decapitation (Versteeg *et al.*, 1979). It should be noted here that the dose of 100 ng per rat, which is much lower than that which causes analgesia, was chosen on the basis of effects of β -LPH fragments in extremely low doses on avoidance behavior (De Wied *et al.*, 1978a, b).

Whereas in the above mentioned studies, since the tyrosine hydroxylase inhibitor α -MPT was used, effects on dopamine utilization rather than on dopamine synthesis were measured, Garcia-Sevilla *et al.* (1978; Ahtee *et al.*, 1978; Magnusson *et al.*, 1979), by measuring the accumulation of DOPA following inhibition of DOPA-decarboxylase with NSD 1015, studied the effects of β -endorphin on striatal catecholamine synthesis. In the experiments in Garcia-Sevilla *et al.* (1978) the rats received the DOPA-decarboxylase inhibitor 10 min after i.c.v. saline or β -endorphin administration and were decapitated 30 min after NSD 1015 injection. Following β -endorphin, given in a dose of 5 or 10 μ g per rat i.c.v. significantly more DOPA was found to have accumulated in the striatum than following saline, indicating an enhanced catecholamine synthesis in this brain region; naloxone antagonized this β -endorphin-induced increase in DOPA accumulation (Garcia-Sevilla *et al.*, 1978).

Effects of β -endorphin on HVA and/or DOPAC concentrations in rat striatum have been studied by Berney and Hornykiewicz (1977), Van Loon and Kim (1977, 1978) and Guidotti *et al.* (1979). The former authors reported that β -endorphin, administered i.c.v. in doses of 1 μ g and up, caused increases in striatal HVA, which were antagonized by naloxone (Berney and Hornykiewicz 1977). Van Loon and Kim found a significantly increased concentration of both HVA and DOPAC after i.c.v. (Van Loon and Kim, 1977) and intracisternal (Van Loon and Kim 1978) administration of 15 μ g β -endorphin. Furthermore, Van Loon and Kim (1978) found that the decrease in striatal HVA and DOPAC concentrations induced by the MAO inhibitor pargyline administered 10 or 40 min after saline or peptide and 20 min prior to decapitation, was greater in rats that had received intracisternal β -endorphin than in rats which had received saline. These findings support the concept that β -endorphin increases striatal dopamine turnover, at least during the first 60 min after its administration (Van Loon and Kim, 1978). The β -endorphin-induced increase in striatal dopamine metabolism appears to be effectuated via opioid receptors: both the increase in striatal HVA and DOPAC levels and the effect on the pargyline-induced decrease in DOPAC level observed in β -endorphin treated rats were found to be counteracted by naloxone (Van Loon and Kim 1978). In a subsequent paper Van Loon *et al.* (1978a) reported that the development of tolerance to intracisternal β -endorphin is associated with a return to normal of dopamine turnover in the striatum. Recently it was shown by Guidotti *et al.* (1979) that striatal HVA concentrations were increased following intrastriatal injection of β -endorphin. Taken together, the available data indicate that β -endorphin is involved in the regulation of striatal dopaminergic activity, possibly via intrastriatal receptors. The effects seem to be dose- and time-dependent.

Like the case for the enkephalins, less is known concerning the effects of endorphins on catecholamine metabolism in other brain regions than the striatum. Izumi *et al.* (1977) reported a decrease in the α -MPT-induced disappearance of dopamine from the mid-brain, dissected according to Glowinski and Iversen (1966), following the i.c.v. administration of 50 μ g β -endorphin, and a tendency in the same direction in the disappearance of noradrenaline from this same region. Fuxe *et al.* (1977) and Schwarcz *et al.* (1979) reported an increased dopamine turnover in the olfactory tubercle, whereas that in the median eminence was found to be decreased. Deyo *et al.* (1979) also observed a decrease in the turnover of dopamine in the median eminence of rats that had received 15 μ g β -endorphin i.c.v. and in the cortex as well. Dopamine turnover in the whole hypothalamus was found to be decreased following intraventricular (20 μ g; Van Vugt *et al.*, 1979) and intracisternal (15 μ g; Van Loon *et al.*, 1980) administration of β -endorphin. Recently, Fuxe *et al.* (1979a) reported that, whereas a dose of approximately 5 μ g of β -endorphin injected i.c.v. only causes a decrease of the dopamine turnover in the medial and the lateral pallisade zones of the median eminence, a dose of approximately 30 μ g also led to an increase of the turnover of catecholamines in the subependymal layer of the median eminence and in the paraventricular nucleus. These findings, according to Fuxe *et al.* (1979a), suggest the existence of opioid receptor mechanisms reducing dopamine turnover and increasing that of noradrenaline in the median eminence. Further support for the modulating influence of opioid peptides on tubero-infundibular dopamine activity has been presented by Gudelsky and Porter (1979). These authors showed that the i.c.v. administration of β -endorphin (10 μ g) or D-Ala²-Met-enkephalimamide (100 μ g) causes an 85–95% reduction in the concentration of dopamine in pituitary stalk plasma, an effect which is abolished following naloxone pretreatment.

Garcia-Sevilla *et al.* (1978) in their study, of which the results concerning the effects on striatal dopamine were mentioned above, also measured the effects of β -endorphin on the accumulation of DOPA following NSD 1015 in limbic structures, hemispheres, diencephalon and the lower brain stem. These authors reported that the effects were qualitatively similar in all brain regions, but that higher doses (10–20 μ g, i.c.v.) were needed to double DOPA accumulation in diencephalon and brain stem, than to do that in the other brain regions. Using a dose of 100 ng per rat i.c.v. Versteeg *et al.* (1979) found that β -endorphin caused a decrease in the α -MPT-induced disappearance of noradrenaline from the ventral part of the nucleus reticularis medullae oblongata, of dopamine in the lateral septal nucleus, and of both amines in the rostral part of the nucleus tractus solitarii; the disappearance of dopamine was increased in the medial septal nucleus and the zona incerta. In this same paper Versteeg *et al.* (1979) also reported the effects of two other β -LPH fragments which have been shown to exert a profound influence on avoidance behavior of rats, cf. α -endorphin (β -LPH_{61–76}) and Des-Tyr¹- γ -endorphin (β -LPH_{62–77}). α -Endorphin, in a dose range of 0.1–3 μ g s.c. and 3–100 ng i.c.v. delays the extinction of pole jumping behavior and is more potent in this respect than β -endorphin (De Wied *et al.*, 1978a). γ -Endorphin and its Des-Tyr¹ analog both have effects on avoidance behavior opposite to those of α -endorphin, i.e. they facilitate the extinction of pole jumping behavior and attenuate passive avoidance behavior (De Wied *et al.*, 1978b). The removal of the N-terminal tyrosine residue of γ -endorphin has been found to cause a complete loss of opiate-like activity and to destroy affinity to opioid receptors (for references see De Wied *et al.*, 1978b), and yields a compound which is even more potent than γ -endorphin on avoidance behavior, significant effects being apparent after doses as low as 30 ng s.c. and 300 pg i.c.v. (De Wied *et al.*, 1978b). It was found (Versteeg *et al.*, 1979) that following the administration of 100 ng i.c.v. the effects of α -endorphin on the α -MPT-induced disappearance of catecholamines were much more widespread throughout the brain than those of β -endorphin, and that without exception, when α -endorphin affected this parameter, it caused a decrease. The psychopharmacological profile of Des-Tyr¹- γ -endorphin resembles that of the classic neuroleptics, though it seems to be more specific (De Wied *et al.*, 1978b; Kovács and De Wied 1978; see also De Kloet and De Wied, 1980). Interestingly, Versteeg *et al.* (1979) found that Des-Tyr¹- γ -endorphin

affected dopamine disappearance from the paraventricular nucleus and the zona incerta in a direction opposite to that induced by α -endorphin, and in a direction also seen after the neuroleptic haloperidol. Until now, however, dose-effect data are lacking. The only data concerning a direct comparison of Des-Tyr¹- γ -endorphin and haloperidol show that, whereas 1 hr after the *in vivo* administration of haloperidol in a dose of 0.62 mg per kg body weight the *in vitro* conversion of ¹⁴C-tyrosine into ¹⁴C-dopamine by caudate slices was increased, this same dose of Des-Tyr¹- γ -endorphin was ineffective (Weinberger *et al.*, 1979). Similar data have been obtained after the i.c.v. administration of these two compounds by Versteeg, Van Heuven-Nolsen and De Wied (unpublished observations). It might be suggested that the effects of Des-Tyr¹- γ -endorphin are less general and that the peptide exerts its effects in a way different from that of haloperidol. It could be that, while haloperidol affects brain dopamine systems via an interaction with dopamine receptors, Des-Tyr¹- γ -endorphin interferes with dopamine receptors indirectly, via, as yet unidentified specific receptors localized on a relatively small population of neurons.

4.2. INTERACTION WITH BRAIN ACETYLCHOLINE

In a series of papers Costa and co-workers have reported the results of their studies of the effects of β -endorphin on regional acetylcholine turnover in rat brain. β -Endorphin, given in a dose of approximately 10 μ g per rat i.c.v., caused a decrease in the turnover of acetylcholine in the hippocampus, nucleus accumbens, globus pallidus and cortex, but, like morphine, not in the caudate nucleus (Moroni *et al.*, 1977a). α -Endorphin, in a dose of approximately 20 μ g had no analgesic activity and did not affect acetylcholine turnover in any of the brain regions studied (Moroni *et al.*, 1977a). It was calculated that the dose of β -endorphin needed to reduce the acetylcholine turnover in the hippocampus by 50% is approximately 0.7 μ g (Moroni *et al.*, 1977a), which makes the peptide several times more potent than morphine (cf. Zsilla *et al.*, 1976). Naltrexone completely prevented both the analgesic action and the effect of β -endorphin on acetylcholine turnover (Moroni *et al.*, 1977a). In subsequent experiments Moroni *et al.* (1979b) found that intraseptal administration of approximately 2.5 μ g of β -endorphin reduced the turnover of acetylcholine in the hippocampus significantly, but not that in the striatum and cortex. The effect of intraseptal β -endorphin on hippocampal acetylcholine turnover was prevented by the i.p. administration of naltrexone (Moroni *et al.*, 1978a). On the basis of these data it was concluded that opioid agonists modulate hippocampal acetylcholine turnover via an interaction with opioid receptors in the septum (Moroni *et al.*, 1977b, 1978a). Thus, the activity of acetylcholine-containing neurons projecting from the septum to the hippocampus might be regulated by peptidergic neurons impinging on the cell bodies of the cholinergic neurons in the septum (Moroni *et al.*, 1977b, 1978a).

In contrast to Moroni *et al.* (1977a), Botticelli and Wurtman (1979) found significant elevations of hippocampal acetylcholine levels following the i.c.v. administration of 10 μ g β -endorphin, with peak effects 30 min after peptide injection. The choline concentration was not changed, while naloxone antagonized the effects of β -endorphin on acetylcholine levels in the hippocampus (Botticelli and Wurtman, 1979). These authors conclude that their data are indicating a decreased utilization of acetylcholine in the hippocampus, which is in keeping with the suggestions of Moroni *et al.* (1978a).

4.3. INTERACTION WITH BRAIN SEROTONIN

β -Endorphin, administered i.c.v. in a dose of 5 μ g per rat, causes an increase in the NSD 1015-induced accumulation of 5-HTP in limbic regions, striatum, diencephalon and in the lower brain stem, but not in the hemisphere (Garcia-Sevilla *et al.*, 1978; Ahtee *et al.*, 1978; Magnusson *et al.*, 1979). The rise in 5-HTP accumulation, however, takes longer to reach peak values than that of DOPA accumulation (Garcia-Sevilla *et al.*, 1978). Van Loon and De Souza (1978), on basis of changes in the concentrations of 5-HT and 5-HIAA and of differences in the changes in the concentration of 5-HIAA following

administration of the MAO inhibitor pargyline, concluded that β -endorphin, given intracisternally in a dose of 15 μg , causes a decrease in the turnover of 5-HT in the hippocampus and an increase in the brain stem and the hypothalamus. The fact that β -endorphin caused a decrease in the pargyline-induced accumulation of 5-HT in all three brain regions (Van Loon and De Souza 1978), leaves some doubt concerning the validity of this conclusion.

4.4. INTERACTION WITH BRAIN GABA

Whereas virtually no data are available concerning possible interactions of ACTH-like peptides and enkephalins with brain GABA, a small number of papers have been published showing that β -endorphin affects GABAergic systems in the brain. Moroni *et al.* (1978b) reported that β -endorphin in a dose of approximately 10 μg , i.c.v., increases the turnover of γ -aminobutyric acid in the substantia nigra and globus pallidus, while it decreases that in the caudate nucleus. GABA turnover was measured by calculating the relative enrichment in ^{13}C of glutamate and GABA following the infusion of uniformly labeled ^{13}C -glucose through the tail vein for 10 min starting 20 min after the i.c.v. injection of either saline or β -endorphin in these brain parts after exposure of the heads of the rats to microwave irradiation (Moroni *et al.*, 1978b). The effects were dose-dependent and could be prevented by naltrexone; no effects were seen on the concentration of either glutamic acid or GABA (Moroni *et al.*, 1978b). Injection directly into the caudatus of approximately 0.7 μg β -endorphin decreased the turnover of GABA in the caudate nucleus, but increased it in the globus pallidus (Moroni *et al.*, 1979). According to these authors it seems likely that activation of opioid receptors located on short GABA-containing neurons in the caudate nucleus causes the increase of GABA turnover in the globus pallidus, due to a decrease in GABAergic inhibition elicited by short GABA neurons on the long GABA neurons linking the caudate nucleus with the globus pallidus (Moroni *et al.*, 1979). Perez de la Mora *et al.* (1979) recently reported evidence that β -endorphin causes an increase in the turnover of GABA in the substantia nigra and the nucleus caudatus. This conclusion was based on the effect of 5 μg β -endorphin, administered i.c.v. on the γ -vinylGABA-induced accumulation of GABA in these brain structures (Perez de la Mora *et al.*, 1979). The finding of an increased GABA turnover in the caudate nucleus contrasts with the observations of Moroni *et al.* (1978b, 1979).

5. CONCLUDING REMARKS

From the foregoing it will be clear that a considerable number of data has been presented supporting the notion that peptides related to ACTH, MSH and β -LPH participate in the regulation of the activity of neuronal systems in the brain containing catecholamines, acetylcholine, serotonin and GABA. Notwithstanding the fact that in many cases the obtained results cannot be compared directly because methods, injection schedules, doses, brain dissection, or even all of them are different, and that in some cases, when a comparison is possible, the results are in obvious contradiction, this rather general conclusion seems to be justified. Since the majority of the available data does not go beyond the simple assessment of the presence or absence, as it may be, of effects of peptide x on parameter y in brain region z, at present a more detailed characterization of peptide effects on neurotransmitter systems appears to be an exception. Because of this, our present knowledge about where in the brain the interactions take place and about what is the neuronal circuitry involved in the interactions is rather limited. Therefore, it seems worthwhile to consider how, in the planning of future research, advantage can be taken of the experimental designs which led to the acquisition of this knowledge and of the potentials of methods other than those used to measure neurotransmitter metabolism.

For reasons mentioned in the introduction, the efforts up till now have concentrated to a great extent on the interaction of peptides with brain catecholamines or, to be more

precise, with striatal dopamine. Taken together, due to these efforts, it now is fairly well established that one of the ways in which endorphins modulate striatal dopamine is via an interaction with opioid receptors localized on the terminals of nigro-striatal dopamine neurons (see Sections 3.1. and 4.1.). Similarly, it has been shown that β -endorphin affects acetylcholine neurotransmission in the septo-hippocampal system following injection into the septum to the same extent as following i.c.v. administration, which suggests that the interaction might be mediated by receptors on the cell bodies of this system rather than on the terminals in the hippocampus (Section 4.2.). Evidently, if such conclusions are to be reached, the experimental design should at least include biochemical measurements following local micro-injection of the peptide in sufficiently small brain regions, as was the case in the two examples given above. Obvious pitfalls in this type of experiments are (a) too large injection volumes, which can easily cause extensive tissue damage and, consequently, leakage of the injected peptide to surrounding brain regions and to the ventricular system, and (b) peptide concentrations which are out of the range of the concentration which can be expected to be reached at the site following i.c.v. administration of the peptide.

From the first example, i.e. that of the interaction of endorphins with striatal dopamine, it is evident that such methods as radioligand binding with appropriate labeled compounds before and after selective lesioning of particular neuronal systems with neurotoxic substances are of great value when data on the location of receptors are to be obtained. There are, however, some complicating factors. In the first place, though the majority of the studied β -LPH-derivatives is supposed to act via opioid receptors, the available evidence clearly indicates the existence of multiple receptors for this class of substances (for recent reviews see Terenius 1980; Herz *et al.*, 1980). Secondly, there is the observation that some of the β -LPH-like peptides act via non-opioid receptors (for references see De Kloet and De Wied 1980; Van Nispen and Greven 1980) and that some of the effects of ACTH-like peptides can be blocked by opiate antagonists (see Gispen and Wiegant 1976; Gispen and Isaacson 1980). As far as ACTH-like peptides are concerned, there is, furthermore, the evidence that the putative brain receptors for these peptides seem to be characterized by a very high affinity and a very low capacity, which makes their detection at present impossible (Witter 1980). Nevertheless it seems likely that these approaches will eventually lead to the unraveling of where the interactions of the various peptides with neurotransmitter systems in the brain take place. Obviously, research along these lines cannot do without the guidance of neuroanatomical data concerning the neuronal pathways involved, while it also needs the support of the results of neuropharmacological experiments.

Whereas the present knowledge concerning the neuronal circuitry involved in peptide action is rather limited, apart from some evidence for how the effects of endorphins on striatal dopamine metabolism are brought about, virtually nothing is known concerning the mechanisms of action of the peptides of the ACTH-MSH-endorphin family. In this respect it should be realized that, in addition to effects on neurotransmitter metabolism in the brain, effects of peptides have been described on other neurochemical parameters as well. Those on cyclic nucleotides, phosphoproteins and protein synthesis have been studied most extensively and are the subject of reviews in this series (Wiegant *et al.*, 1980; Dunn 1980). It is not known whether these effects and those discussed here are part of a chain of events triggered by receptor activation and, if so, how these events are associated. The possible mechanisms of action of neuropeptides have been the subject of discussion in a number of reviews in recent years (e.g. Dunn and Gispen 1977; Walter and Hoffman 1977; Gispen *et al.*, 1977; Barchas *et al.*, 1978; Barker 1978; Iversen *et al.*, 1978; De Wied and Versteeg 1979). It is not the place here to summarize the suggestions put forward in these reviews, which ultimately are all models based on the effects that have been observed of the various peptides and on what is known concerning the mechanisms of action of the classical neuroregulators. Obviously, progress towards a better understanding of how the peptides related to ACTH, MSH and β -LPH act will depend on the concerted efforts of various neurochemical subdisciplines.

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