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EFFECTS OF ADRENALECTOMY AND HYPOPHYSECTOMY ON ENKEPHALIN CONTENT OF THE RAT HYPOTHALAMUS

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1 Hypothalamic content of enkephalin in rats has been measured by bioassay against methionine-enkephalin on field-stimulated mouse vas deferens after acid extraction and purification using Amberlite XAD-2 resin.

2 Surgical stress lowered hypothalamic enkephalin content initially but 6 days after operation the content was higher in sham-operated than in adrenalectomized animals.

3 Corticosteroid replacement therapy showed that hypothalamic enkephalin content was not related directly to circulating corticosteroid levels and that it was increased by the stress of handling and injection.

4 Hypothalamic enkephalin content of rats that had undergone hypophysectomy 11 days earlier did not differ from that of intact animals but surgical stress, which lowered content in intact rats and had no effect after adrenalectomy, produced a rise in content in these hypophysectomized animals.

5 The ingestion of 0.9% saline, in place of water, by sham-adrenalectomized rats altered the effect of surgical stress on hypothalamic enkephalin content without affecting the resting levels.

6 Of extracts from adrenalectomized rats, 10% contained a substance that behaved atypically on the mouse vas deferens, showing a slower onset and offset of action. The mol. wt. of the substance and sensitivity to naloxone reversal appeared to be similar to enkephalin, but it was resistant to carboxypeptidase-A and protease treatment.

7 It is concluded that the enkephalin content of the hypothalamus is affected by activity in the hypothalamus–pituitary–adrenal system but that it is not related in a simple manner to the levels of corticotrophin releasing hormone, corticotrophin or corticosteroids.

Introduction

Enkephalins appear to be neurotransmitters in the brain (Hughes, 1979) and their involvement in the control of the endocrine system is suggested by the ability of morphine to affect anterior pituitary function (George, 1971). There is direct evidence that methionine-enkephalin is capable of increasing plasma concentrations of prolactin (Lien, Fenichel, Garsky, Sarantakis & Grant, 1976) and growth hormone (Dupont, Cusan, Garson, Labrie & Li, 1977) whilst it lowers those of luteinizing hormone and thyrotrophin (Bruni, Van Vugt, Marshall & Meites, 1977). That enkephalins might also be involved in the hypothalamus–pituitary–adrenal (HPA) system is suggested by the enhancement of the corticosterone response to ether stress produced by the intracerebroventricular administration of methionine-enkephalin to mice (Gibson, Ginsburg, Hall & Hart, 1979b).

Morphine is considered to affect the HPA system by an action in the hypothalamus (George & Way, 1959; Lotti, Kokka & George, 1969). The high content of opioid receptors (Kuhar, Pert & Snyder, 1973) and enkephalin-like immunoreactive material (Elde, Hökfelt, Johanssen & Terenius, 1976) in this region make it a likely site for the action of enkephalin on the HPA system. For this reason we have measured the enkephalin content of the hypothalamus of the rat with the HPA system in different functional states. Some aspects of this work have already been published (Gibson, Ginsburg, Hall, Hart & Kitchen, 1978; Gibson, Ginsburg, Hart & Kitchen, 1979).

Methods

Animals and operative procedures

Male Wistar albino rats (Anglia Laboratory Animals)

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weighing 175 to 275 g were housed in a constant light cycle room (lights on 07 h 00 min to 21 h 00 min) at 22°C. Hypophysectomized and sham-hypophysectomized animals were supplied by Charles River Laboratories 7 days after operation and experimental procedures begun on the 11th day. Hypophysectomy and bilateral adrenalectomy were performed under ether anaesthesia by the parapharyngeal and dorsal midline approaches respectively. Success of operative procedure was monitored by lack of weight gain, post-mortem examination of hypophysectomized animals and where appropriate, plasma corticosterone levels. Unless otherwise stated, adrenalectomized and sham-adrenalectomized rats were maintained on 0.9% w/v sodium chloride solution (saline). Hypophysectomized, and half of the sham-hypophysectomized animals, were given 5% glucose solution whilst the other half received tap water. All animals were allowed to acclimatize to laboratory conditions for at least 45 min before killing; experimental and operative procedures were performed between 09 h 30 min and 11 h 30 min.

Administration of drugs

Dexamethasone (5×10^{-5} M) was administered to rats in their drinking water over 24 h. Approximately 3.5 mg/kg was ingested during this period. Corticosterone (10 mg/kg, s.c. in vegetable oil) was given to unoperated rats 24 h before they were killed. Corticosterone replacement therapy of adrenalectomized animals was achieved by means of two protocols: (i) a single daily dose of 10 mg/kg (s.c.) in vegetable oil and (ii) divided daily doses of 5 mg/kg at 09 h 00 min and 15 mg/kg at 17 h 00 min (s.c.) in an aqueous suspension.

Tissue extraction and bioassay

Hypothalamic blocks were dissected as described by Glowinski & Iversen (1966) and enkephalin extracted with 0.1 M HCl (5 to 8 ml), homogenized and centrifuged (20,000 *g* for 10 min), desalted using Amberlite XAD-2 resin and eluted with 90% methanol (50 ml) essentially as described by Hughes, Kosterlitz & Smith (1977). Dried residues were taken up in Mg^{2+}-free Krebs (200 µl) and 50 µl aliquots assayed against methionine enkephalin for opioid peptide activity on the field stimulated mouse vas deferens (Hughes *et al.*, 1977). Bioassay parameters are described elsewhere (Hart, Kitchen & Waddell, 1979).

Chromatography and enzyme studies

Apparent molecular weight determinations were carried out by means of Sephadex G-15 gel filtration chromatography (Hughes *et al.*, 1977). Dried G-15 residues were investigated for susceptibility to enzymatic hydrolysis. Fractions were dissolved in 0.1 ml Krebs solution containing carboxypeptidase A (20 µg/ml) or protease (150 µg/ml), incubated at 37°C for 30 min and assayed immediately. Trypsin (100 µg/ml) hydrolysis was carried out as described by Hughes (1975).

Corticosterone assay

Trunk blood was collected into heparinised tubes and plasma corticosterone assayed fluorimetrically by a modification of the method of Zenker & Bernstein (1958).

Materials

Corticosterone, dexamethasone, trypsin (E.C. 3.4.4.4., Type III) carboxypeptidase A (E.C. 3.4.2.1., Type I) and protease (Type VI) were obtained from Sigma and methionine-enkephalin from Calbiochem. Leucine-enkephalin was a gift from Dr T.W. Smith (Wellcome, Beckenham) and β-endorphin from Dr D.G. Smyth (NIMR, Mill Hill).

Results

Preliminary experiments confirmed the observations of Hughes *et al.*, (1977) that more than 92% of the opioid-like activity from the XAD-2 columns co-chromatographed with the enkephalins on a Sephadex G-15 gel. Addition of β-endorphin (2 µg) to the 0.1 M HCl used for homogenization did not result in additional enkephalin activity in the final extract. Figures for enkephalin content are uncorrected for recovery which was 96.1 ± 5.5% ($n = 9$).

Hypothalamic enkephalin content of untreated rats

Long-term monitoring of hypothalamic content of enkephalin in untreated rats revealed a significant change in levels. It was not possible to relate this to any change in the source or treatment of animals, or to an alteration in the extraction or bioassay procedures. The enkephalin content of hypothalami from appropriate control animals was therefore determined during each set of experiments. Within the Series I experiments it was found that there was no significant difference between the enkephalin levels in hypothalami from male and female rats (Table 1).

Hypothalami were removed from rats at different times during the day but although the enkephalin content rose between 09 h 30 min and 16 h 00 min the change was not significant. The results in Table 1 show that there was no correlation between enkepha-

lin content and the diurnal changes in plasma corticosterone.

Hypothalamic enkephalin content of adrenalectomized rats

Two sets of experiments, shown in relation to their respective control values, are presented as Series I and Series II in Figure 1. The time course of changes in hypothalamic enkephalin levels in adrenalectomized and sham-adrenalectomized rats is shown. The pattern of changes was similar in each series with an initial fall in content occurring in both adrenalectomized and sham-operated animals. This initial fall was due to surgery and not the ether anaesthetic because exposure to ether vapour for 10 min without surgery produced no significant change in content measured 24 h later (Table 4). Six days after operation the hypothalamic enkephalin content in sham-operated rats was significantly greater than that in the adrenalectomized whilst by 11 days, levels had returned to pre-operation values in both groups.

The changes which occurred in the hypothalamus were not matched throughout the brain since 24 h after adrenalectomy there was no significant change in enkephalin levels in extracts of brain tissue which excluded the hypothalamus and cerebellum (control 807 ± 43, $n = 6$; adrenalectomized 706 ± 73; $n = 6$, $P > 0.2$).

Effect of exogenous corticosteroids on hypothalamic enkephalin levels

The effects of exogenous corticosteroids were investigated for it seemed likely that the fall in enkephalin content after operation (Figure 1) might be influenced by the increase in plasma corticosterone resulting from the surgical stress. Two routes of administration were used and with both the hypothalamic content of enkephalin increased (Table 2). The elevation after dexamethasone administration in the drinking water was presumably due entirely to the absorbed steroid whereas the response with oil alone (Table 2) indicated that the injection stress and absorbed corticosterone both contributed to the change in hypothalamic enkephalin content observed after the subcutaneous injection of corticosterone.

Six days after operation the enkephalin content was higher in hypothalami from sham-operated than adrenalectomized rats and an attempt was made to see whether replacement therapy raised the hypothalamic enkephalin content of the adrenalectomized animals. The results presented in Figure 2 show that the daily subcutaneous injection of corticosterone in oil did not give satisfactory replacement. However, in spite of the low plasma corticosterone, the hypothalamic enkephalin content reached the level in sham-operated animals due partly to the stress of the injections. When corticosterone was injected twice daily (5 mg/kg and 15 mg/kg, in water) to adrenalectomized rats, plasma corticosterone concentrations returned to control levels and the enkephalin content of the hypothalamus was similar to that of sham-operated rats. This rise in hypothalamic enkephalin could not be attributed to the replacement therapy because a similar change occurred in adrenalectomized rats which received two daily subcutaneous injections of water and in which plasma corticosterone concentrations remained depressed (Figure 2).

Effect of laparotomy stress in intact and adrenalectomized rats

Hypothalamic enkephalin content fell after the major stress of sham-adrenalectomy (laparotomy) whilst the administration of corticosteroid, or the minor stress of subcutaneous injection, caused enkephalin content to rise. To determine the role of circulating cortico-

Table 1 Hypothalamic enkephalin content and plasma corticosterone (Cs) concentrations in untreated rats

Animals and time of sampling	*No. of animals*	*Enkephalin content**	*Plasma Cs* (ng/ml)
Female 09 h 30 min–10 h 30 min	5	631 ± 63	
Male (Series I)			
09 h 30 min–10 h 30 min	10	671 ± 30	
Male (Series II)			
09 h 30 min–10 h 30 min	21	940 ± 63	
09 h 30 min	4	888 ± 97	221 ± 40
12 h 30 min	4	946 ± 82	290 ± 13
16 h 00 min	4	1059 ± 37	199 ± 47

Values are mean $\pm$ s.e. mean.
* ng methionine enkephalin equivalents per g wet wt. hypothalamus.

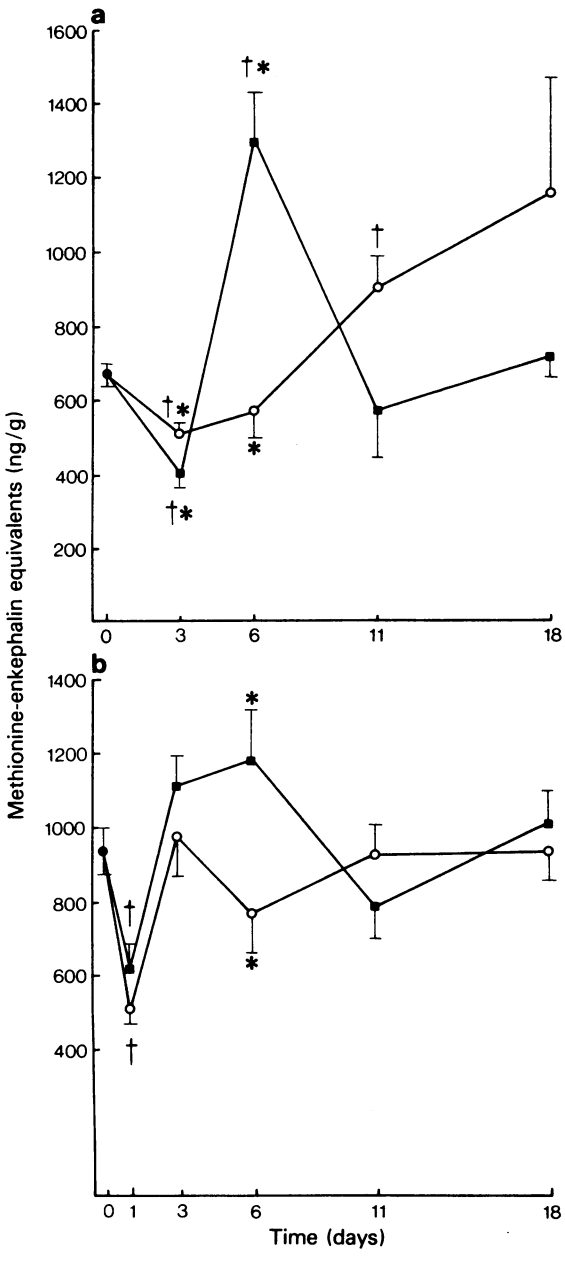

Figure 1 Hypothalamic enkephalin content in rats after adrenalectomy (○) and sham-adrenalectomy (■): (a) is Series I and (b) is Series II. Each point is mean result from at least five animals and vertical lines denote s.e. mean. † $P < 0.05$ control vs operated; * $P < 0.05$ sham vs adrenalectomy.

sterone in the response to laparotomy stress the operation was performed 18 days after adrenalectomy or

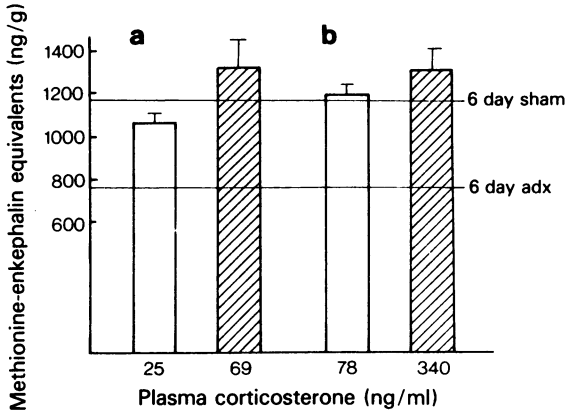

Figure 2 Hypothalamic enkephalin content and plasma corticosterone concentration in adrenalectomized rats after corticosteroid replacement therapy (hatched columns) or vehicle alone (open columns). (a) Single daily dose of 10 mg/kg (s.c.) in vegetable oil; (b) 5 mg/kg at 09 h 00 min and 15 m/kg at 17 h 00 min (s.c.) in an aqueous suspension. Six animals in each group, vertical lines denote s.e. mean. Horizontal lines denote enkephalin content in 6 day adrenalectomized rats (6 day adx 765 ng/g ± 103) and in 6 day sham-adrenalectomized rats (6 day sham, 1180 ng/g ± 136).

sham-adrenalectomy, i.e. at a time when the enkephalin content of the hypothalamus had returned to normal. The results presented in Table 3 show that laparotomy stress did not alter hypothalamic enkephalin levels in adrenalectomized rats but that in sham-adrenalectomized animals the stress caused a significant increase in enkephalin content in contrast to the decrease seen in untreated rats (Figure 1). These results suggested that the first laparotomy affected the enkephalin response to a second operation 18 days later. The other difference between untreated and sham-operated animals was that the latter had been

Table 2 The effect of exogenous corticosteroids on hypothalamic enkephalin content

Treatment	*No. of animals*	*Enkephalin content* (ng/g)
Control	10	842 ± 75
Dexamethasone*	6	1179 ± 103
Corticosterone†	6	1432 ± 96
Corticosterone vehicle	6	1070 ± 135

Values are mean ± s.e. mean.
* Dexamethasone 20 μg/ml in drinking water for 24 h.
†Subcutaneous injection of corticosterone 2 mg in oil 24 h before estimation.

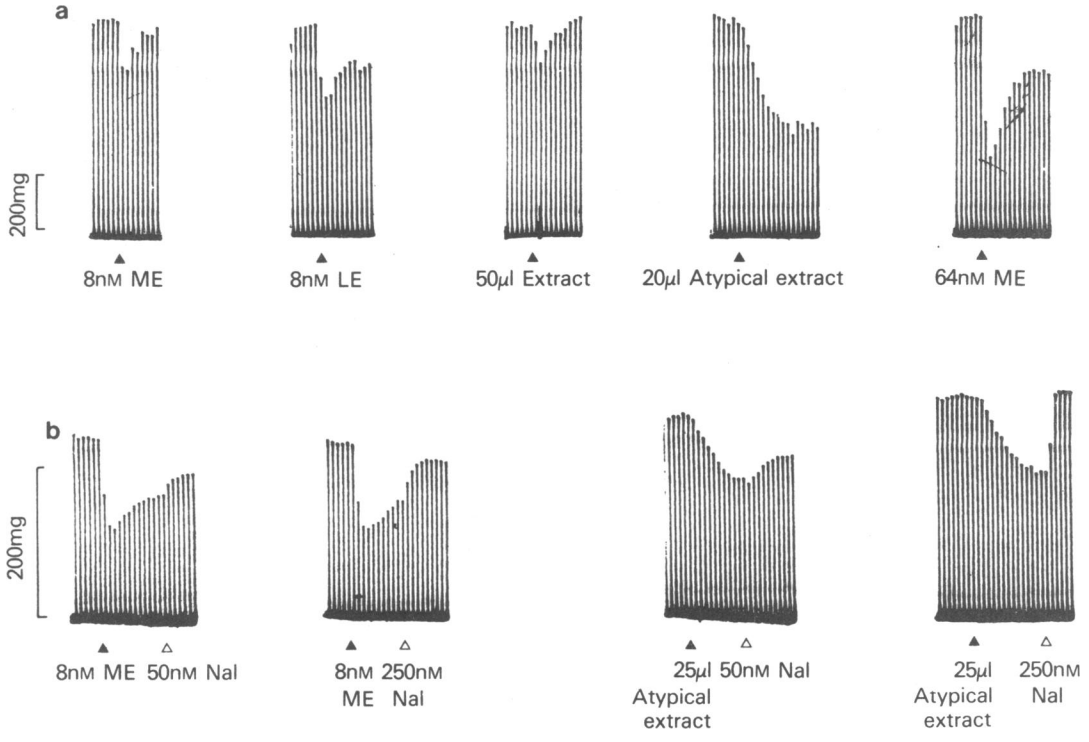

Figure 3 Isometric contractions of the field-stimulated mouse vas deferens. (a) Comparison of the inhibition produced by methionine-enkephalin (ME), leucine-enkephalin (LE), an enkephalin-like extract and an atypical extract. Stimulation 0.1 Hz, 1 ms, 530 mA. (b) Sensitivity of methionine-enkephalin and atypical extract to naloxone (Nal). Stimulation 0.1 Hz, 1 ms, 170 mA.

maintained on saline in a similar manner to the adrenalectomized rats. When sham-operated rats were allowed access to tap water for 18 days a subsequent laparotomy caused a drop in hypothalamic enkephalin content (Table 3) similar to that in untreated animals. Thus although the ingestion of saline in place of water for 18 days did not affect the content of enkephalin in the hypothalami of sham-operated rats, it did alter the stress-induced change in content.

It was also shown that three days after sham-adrenalectomy there was no significant difference between the hypothalamic enkephalin content of rats allowed access to tap water (452 ± 65, $n = 6$) and those drinking saline (397 ± 32, $n = 9$; $P > 0.4$).

Table 3 Hypothalamic enkephalin levels measured 24 h after laparotomy in rats adrenalectomized, or sham-adrenalectomized 18 days previously

Treatment	*No. of animals*	*Enkephalin content* (ng/g)
18 day adrenalectomized (adx)	4	827 ± 156
18 day adx + laparotomy	6	825 ± 71
18 day sham-adx (saline)	4	971 ± 65
18 day sham-adx (saline) + laparotomy	6	1364 ± 72
18 day sham-adx (water)	4	867 ± 72
18 day sham-adx (water) + laparotomy	5	631 ± 45

Values are mean $\pm$ s.e. mean.

Hypothalamic extracts which produced atypical responses on the mouse vas deferens preparation

Nine out of the 89 hypothalmic extracts from adrenalectomized rats produced a prolonged inhibition of the twitch of the mouse vas deferens. Figure 3 shows that the inhibition produced by methionine- or leucine-enkephalin, or by a typical hypothalamic extract, developed rapidly and was maximal for only 20 s. In contrast, the atypical response developed over about 90 s and was maintained until the preparation was washed. This atypical response was not seen in any of

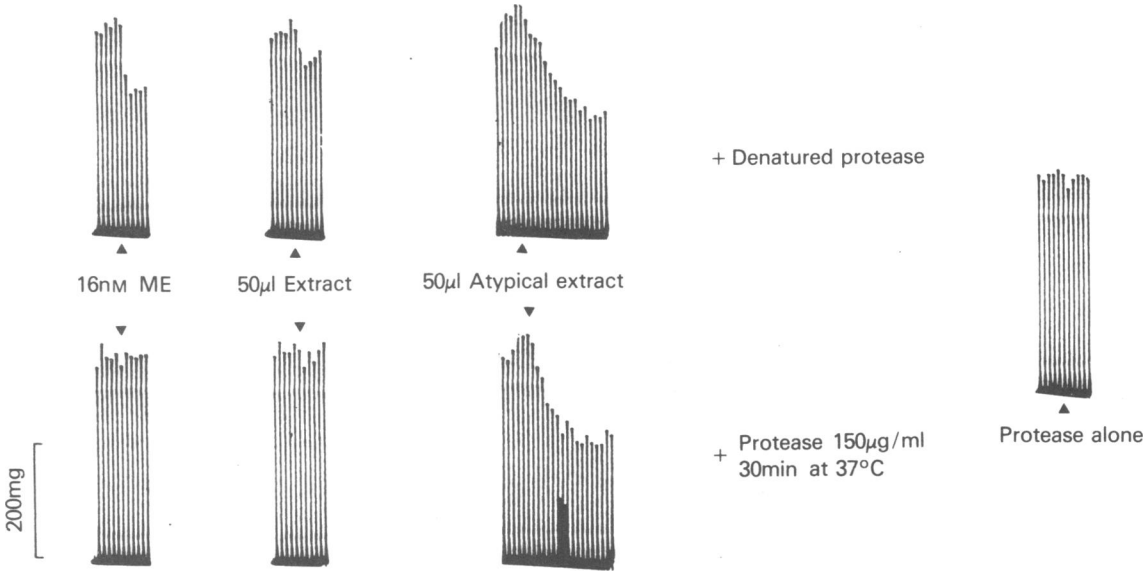

Figure 4 Isometric contractions of the field-stimulated mouse vas deferens. Effect of protease and denatured protease on the response to methionine-enkephalin (ME), enkephalin-like extract and atypical extract. Stimulation 0.1 Hz, 1 ms, 530 mA.

the 180 extracts from intact, sham-operated or hypophysectomized animals.

Each of the extracts that gave atypical responses was inhibited by naloxone (900 nM), and the sensitivity of the atypical response to low concentrations of naloxone (50 and 250 nM) was similar to that of methionine-enkephalin (Figure 3).

Extracts producing atypical responses were present in Series I and Series II experiments, none occurred before 6 days and the majority occurred 11 days after adrenalectomy. The values for opioid activity in these extracts were not included in Figure 1 because not only was the opioid activity clearly not due to enkephalin but in several extracts the concentration of opioid material, expressed in terms of methionine-enkephalin equivalents, was extremely high (up to 60 μg/g). Further analysis of this material showed that it co-chomatographed with methionine- and leucine-enkephalin on a Sephadex G-15 column indicating an equivalent molecular weight. It differed from the two

Table 4 Hypothalamic enkephalin content and plasma corticosterone (Cs) concentrations, measured 24 h after treatment indicated, in rats hypophysectomized or sham-hypophysectomized, 11 days previously

Treatment	*No. of animals*	*Enkephalin content* (ng/g)	*Plasma Cs* (ng/ml)
Intact	8	1244 ± 90	237 ± 36
+ laparotomy	6	822 ± 49†	
+ 10 min ether	5	1344 ± 109	
Hypophysectomy	6	973 ± 125	91 ± 18
+ adrenalectomy	10	1334 ± 92*	114 ± 27
+ laparotomy	6	1528 ± 79†	191 ± 18†
+ dexamethasone	6	1246 ± 45	89 ± 31
Sham-hypophysectomy	5	1281 ± 147	211 ± 27
+ adrenalectomy	5	1637 ± 105	160 ± 24
+ laparotomy	6	1389 ± 142	283 ± 49

Values are mean ± s.e. mean.
t test, * $P < 0.05$, † $P < 0.01$ vs relevant control.

peptides by its resistance to breakdown by carboxypeptidase-A and the non-specific peptidase, protease (Figure 4). Trypsin hydrolysis was also ineffective in altering the activity of the atypical response.

Effect of hypophysectomy, and of surgical stress in hypophysectomized rats, on hypothalamic enkephalin levels

All the hypophysectomized rats were maintained on glucose solution for 11 days; adrenalectomized animals were then given saline in addition to glucose whereas those sham-adrenalectomized remained on glucose. Sham-hypophysectomized rats were offered either glucose or water to drink; the results were found not to be dependent on the drinking solution.

Eleven days after hypophysectomy, or sham-hypophysectomy, the levels of enkephalin in the hypothalamus were not significantly different from those of untreated rats (Table 4) although the content of hypophysectomized animals was 24% lower than that of the sham-operated animals. When the 11 day hypophysectomized rats were adrenalectomized, and hypothalamic enkephalin content measured 24 h later, there was a significant increase in enkephalin levels ($P < 0.05$, Table 4) in contrast to the fall which occurred in untreated animals (Figure 1). Similarly, content was significantly higher 24 h after laparotomy in hypophysectomized rats whereas the similar operation in adrenalectomized animals did not change enkephalin levels (Table 3) and in sham-adrenalectomized or untreated animals produced a fall in content (Figure 1 and Table 4). The administration of dexamethasone in drinking water for 24 h significantly increased the hypothalamic enkephalin content of intact rats (Table 2) whilst in hypophysectomized animals the 28% increase produced by dexamethasone was not significant.

In sham-hypophysectomized rats, enkephalin content was significantly increased 24 h after adrenalectomy but laparotomy did not cause a significant change.

In this series of experiments plasma corticosterone levels were also measured. The concentration in 11 day hypophysectomized rats was unaffected by adrenalectomy or dexamethasone treatment but was elevated by laparotomy stress. (Table 4).

Discussion

This study has shown that the hypothalamic content of enkephalin in the rat is affected by changes in HPA activity. Changes in content do not parallel the diurnal changes in circulating corticosterone concentrations and there appears to be no direct relationship between hypothalamic enkephalin content and simultaneous plasma corticosterone concentrations.

The fall in hypothalamic enkephalin content was seen 24 h and 72 h after both adrenalectomy and sham-adrenalectomy; this was probably due to the stress of surgery since exposure to anaesthetic alone caused no change in enkephalin content. In contrast, most criteria of HPA activity measured after sham-adrenalectomy indicate changes of shorter duration. For example, plasma corticotrophin (ACTH) and corticosterone concentrations return to normal values 80 min after sham-adrenalectomy whilst plasma corticosterone is significantly depressed 2.5 h after adrenalectomy (Buckingham & Hodges, 1974; Buckingham, 1979). On the other hand, it has been reported (van Dijk, van Wimersma Greidanus, de Kloet & de Wied, 1979) that ACTH levels in hypothalami of rats are decreased 3 days after adrenalectomy or sham-adrenalectomy, a time-course similar to that found here for changes in hypothalamic enkephalin. This parallelism of the changes in enkephalin and ACTH in hypothalamus may arise by virtue of their origin from a common precursor.

The only stage at which the hypothalamic content of enkephalin differs between adrenalectomized and sham-adrenalectomized rats is at 6 days after operation, when the enkephalin is lower in the adrenalectomized animals. At this time after adrenalectomy, hypothalamic CRH, and ACTH in pituitary and plasma, are elevated whilst plasma corticosterone is low (Buckingham & Hodges, 1974; Buckingham, 1979) but, in contrast to the decrease in hypothalamic enkephalin content, these changes persist.

It has been shown (Buckingham & Hodges, 1977; Buckingham, 1979) that hypothalamic CRH and pituitary ACTH are reduced by betamethasone but increased by ether stress. However, we found that both minor stress (handling and injection) and dexamethasone treatment increased hypothalamic enkephalin content. These observations emphasise the difficulty in relating hypothalamic enkephalin content to activity in the HPA system and suggest that hypothalamic enkephalin content is influenced by stress-induced activity from higher centres independently of any influence, direct or indirect, arising from the HPA axis.

A clue to the complex relationship between hypothalamic enkephalin content and HPA activity is obtained from a comparison of the effects of laparotomy stress in previously untreated rats, in 18 day adrenalectomized animals and in water-drinking 18 day sham-adrenalectomized animals. In the untreated and sham-operated animals, enkephalin content fell whereas in the adrenalectomized rats, the stress of laparotomy had no effect. However, this fall in hypothalamic enkephalin content after surgical stress appears to depend upon factors other than, or in

addition to, the presence of corticosteroids because laparotomy in hypophysectomized rats caused not a fall, but an increase of enkephalin in the hypothalamus. The observation that laparotomy in the hypophysectomized rats raises plasma corticosterone agrees with the recent report of Ventura, Gonzalo & Goni (1977) of elevated plasma corticosterone following immobilisation stress in hypophysectomised rats. The post-mortem finding that hypophysectomy was complete in these experiments suggest that extrapituitary factors contribute to the steroid response.

An unexpected observation was that 18 day sham-adrenalectomized rats responded to laparotomy stress with a fall in hypothalamic enkephalin content only when they had been maintained on tap water; the consumption of saline reversed the enkephalin response to stress but did not affect resting content. It is to be presumed that drinking saline in these intact animals modified the secretion of aldosterone and vasopressin in the resting state and possibly also the secretion of these hormones during or following surgical stress. Vasopressin is connected with both enkephalins and the HPA system since it is believed to act synergistically with CRH (Buckingham & Leach, 1979) and it is released from the neurohypophysis by leucine-enkephalin (Bisset, Chowdrey & Feldberg, 1978).

The use of bioassay for enkephalin enabled the presence of a substance which behaved atypically to be recognised in hypothalamic extracts from adrenalectomized rats. The substance has been partially characterized; it appears to have a molecular weight similar to that of enkephalin, it is resistant to procedures that normally inactivate peptides and it behaves like a lipid soluble opioid on the mouse isolated vas deferens. Compounds with similar properties have been reported during the search for endogenous opioids but their structures are not known. Pert, Pert & Tallman (1976) reported the presence in blood of 'anodynin' which appeared to be derived from the pituitary and was more resistant than other endogenous opioids to degradation by brain enzymes. Of greater interest are the materials isolated from blood and urine by Wüster, Loth & Schulz (1978). These differ from enkephalin and endorphin by the time-course of their opioid-like actions on isolated tissues, by their complete resistance to pronase P treatment and their considerable resistance to acid hydrolysis. Thus, in several respects the substances identified by Wüster *et al.* (1978) resemble that reported in the present study whereas the morphine-like compound described by Gintzler, Gershon & Spector (1978), although resistant to peptidases, is not naloxone-sensitive.

An involvement of endogenous opioids in endocrine function is predicted from a knowledge of the pharmacology of morphine although it cannot be assumed that morphine's effects are due to an agonist action on opioid receptors (Gibson, Ginsburg, Hall & Hart, 1979a). Considerable progress has been made in elucidating the action of endogenous opioids on the secretion of prolactin and luteinizing hormone, where enkephalin appears to block the actions of dopamine (Enjalbert, Ruberg, Arancibia, Priam & Kordon, 1979; Rotsztejn, Drouva, Pattov & Kordon, 1978), but the involvement with the HPA system is much less clear. The present study demonstrates that such an involvement exists and that hypothalamic enkephalin content is not related in a simple fashion to the level of CRH, ACTH or corticosteroids. Content studies do not permit a distinction to be made between synthesis and release but as a hypothesis it is suggested that corticosteroids have a permissive role in the synthesis of enkephalin and that a factor from the pituitary also may be involved in the regulation of enkephalin release.

One interesting question arising from these results is the relevance of the HPA system to the development of opioid tolerance and dependence. Recently Jacquet (1978) has suggested that ACTH and β-endorphin may form part of an integrated neuromodulatory system and that opioid abstinence symptoms may result from altered interactions between these two peptides following chronic opioid administration. In addition some of the peripheral manifestations of morphine withdrawal have been linked with the raised plasma corticosteroid concentrations which occur at this time (Gibson & Pollock, 1975), although Wei (1973) found no evidence for an HPA involvement in opioid tolerance and dependence. Lee Peng (1973), after studying some of the effects of opioids on the actions of corticosteroids, suggested that morphine 'simulates a hormone' and becomes indispensable in a newly established hormone balance. The present results suggesting a role for the endogenous opioid peptides in the HPA system together with those of Jacquet (1978) again raise the possiblity that the widespread disturbances observed during opioid withdrawal may be related to prolonged alterations in normal endocrine function.

It is concluded that enkephalin in the hypothalamus of the rat is involved in the regulation of HPA activity but the determination of its precise role, and relationship with the pituitary endorphins, is dependent upon future studies of the factors affecting the turnover of enkephalin as well as its mode and site of action in the hypothalamus.

I.K. is an M.R.C. student.

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