Maturitas, 6 (1984) 217–223
Elsevier

MAT 00320

The neuropeptide concept

D. de Wied

Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, The Netherlands

(Received 6 June 1984; accepted after revision 23 July 1984)

(Key words: Neuropeptides, Pharmacology, Endorphins, ACTH, ACTH (4–10), Vasopressin, Central nervous system)

Introduction

In 1960/61 it was found that the release of adrenocorticotrophic hormone (ACTH) in response to emotional, but not to somatic or systemic stress, was diminished in rats in which the vasopressin storage organ, the posterior intermediate lobe of the rat pituitary, had been surgically removed [1,2]. Smelik [1] used adrenal ascorbid acid depletion and De Wied [2] plasma corticosterone levels to determine the release of ACTH from the anterior pituitary. Long-term treatment of posterior lobectomized rats with a long-acting vasopressin preparation restored not only the water metabolism (the rats had mild diabetes insipidus), but also the pituitary–adrenal response to emotional stress. This suggested that vasopressin was involved in the release of ACTH from the anterior pituitary, possibly as the result of a behavioural incompetence in transducing the influence of emotional stress from higher brain centres to the hypothalamic pituitary complex [2]. This prompted the author, at the end of the fifties, to study the behaviour of posterior lobectomized rats. Avoidance behaviour was chosen because of its association with stress and emotion.

Research and Findings

Posterior lobectomized rats appeared to learn to avoid as successfully as sham-operated control animals, but they lost the response much faster. The posterior lobectomized rats were in fact behaviourally abnormal. It was found [3] that this abnormality could be corrected not only by long-term treatment with long-acting pitressin or purified (lysine^8) vasopressin (LVP) but also with ACTH and α-MSH

Correspondence to: Professor Dr. D. de Wied, Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands.

(α-melanocyte-stimulating hormone). The latter finding was puzzling, but there was a difference. In order to be active, ACTH had to be given during the extinction period, whereas pitressin injection normalized extinction irrespective of whether it was given during the training or the extinction period. In contrast to posterior lobectomized rats, adenohypophysectomized animals acquired the response less well than sham-operated controls. Long-term treatment with ACTH normalized avoidance acquisition in adenohypophysectomized rats, suggesting that the absence of ACTH caused the deficiency. Totally hypophysectomized rats had the same deficiency as adenohypophysectomized animals [4]. Two findings suggested that the effect of ACTH was not mediated by the adrenal cortex. Dexamethasone, a potent glucocorticosteroid, was not able to restore avoidance learning in hypophysectomized rats, and α-MSH, which is structurally related to ACTH but rather ineffective in activating the adrenal cortex, had the same beneficial effect as ACTH on avoidance learning in such rats [5].

In the sixties, the opportunity for structure-activity relationship studies had become possible as a result of the advances in peptide chemistry [6]. The N-terminal of ACTH contained the information essential for avoidance learning. ACTH(4–10) was the shortest peptide that was as potent as the parent molecule in restoring avoidance learning in hypophysectomized rats [5]. The fact that ACTH(4–10) was present in ACTH as well as in α-MSH and β-MSH explained why the latter hormones had the same influence on avoidance behaviour as ACTH. It was assumed that ACTH and the peptides related to it acted in the brain and it was postulated that pituitary peptides that were related to ACTH but did not have metabolic or peripheral endocrine effects could play a role in the formation of conditioned and other adaptive responses. The pituitary gland seemed a likely site for the production of such peptides having neurogenic or neurotrophic activities which, for example, influenced central nervous structures involved in motivational, learning or memory processes [5].

Following the studies in hypophysectomized rats, further experiments were performed with intact rats. ACTH and related peptides did not stimulate acquisition as much in intact rats as in hypophysectomized rats. However, they delayed extinction of shuttle box or pole-jumping avoidance behaviour and facilitated passive avoidance behaviour. They also delayed extinction of food-motivated behaviour in hungry rats, sexually motivated behaviour and conditioned taste aversion. The findings were reviewed by De Wied and Jolles in 1982 [7]. The effects observed were interpreted as an influence on motivation, attention and vigilance. Under the influence of ACTH-like peptides the animal pays more attention to the specific cues which elicit the behavioural responses.

The difference between the effect of vasopressin and that of ACTH and related peptides on avoidance behaviour observed in posterior lobectomized rats was also seen in intact animals [8]. Treatment with long-acting α-MSH or vasopressin injected subcutaneously every other day during the extinction period inhibited the extinction of shuttle box avoidance behaviour. However, if these preparations were given during the learning period and the treatment was stopped when the extinction trials were started, extinction was inhibited only in animals which had been treated with

long-acting vasopressin. Moreover, if the rats were subjected to a second extinction period 21 days after the end of the first one, the rats which had been treated with the vasopressin preparation during either the acquisition or the extinction period still retained the avoidance response. It was inferred that vasopressin maintains a conditioned avoidance response irrespective of the time of treatment, while α-MSH inhibits extinction during the period of treatment only. This means that there is a basic difference in the mechanisms by which vasopressin and ACTH-like peptides affect conditioned avoidance behaviour. The same contrast between the effects of the peptides was found in hypophysectomized rats by Bohus et al. [9]. The animals were treated with either ACTH(4–10) or vasopressin during the first week of avoidance learning in the shuttle box. Both treatments normalize the acquisition of avoidance behaviour by hypophysectomized rats. After discontinuation of the treatment, avoidance learning in rats treated with ACTH(4–10) deteriorated despite shock punishment if the animals failed to respond, whereas vasopressin-treated rats maintained a high level of performance after discontinuation of the treatment. This long-term effect of vasopressin was interpreted as an effect on memory processes and the short-term effect of ACTH(4–10) as a motivational influence.

Whether or not pituitary hormones such as ACTH gained access to the brain was a matter of debate for many years. The neurohypophyseal hormones vasopressin and oxytocin, which are formed in the paraventricular and supraoptic nuclei of the hypothalamus, might be distributed throughout the brain, possibly via the cerebrospinal fluid following discharge into the third ventricle. Some evidence for this from immunohistochemical studies had been published [10]. However, for ACTH or α-MSH to reach the brain a retrograde transport from the pituitary gland had to be assumed, this having been suggested for many years. Studies by Porter's group in the USA demonstrated retrograde flow, since large amounts of pituitary hormones were found in the portal vessel system, as reported by Oliver et al. in 1977 [11]. Bergland and Page [12] obtained evidence in 1978 of retrograde flow using the scanning electron microscope to examine vascular casts of the portal vessel system. Evidence has also been found of the retrograde flow of an ACTH-like peptide and neurotensin following microinjection into the anterior pituitary gland of the rat. Mezey et al. [13] and Dorsa et al. [14] reported that the flow of these peptides could be temporarily disrupted by stalk section.

At the time the studies on retrograde transport of pituitary hormones were being conducted, the presence of opiate receptors and opiate-like material in the brain of rats had already been discovered. In 1975, using the guinea pig ileum for bioassay, Hughes et al. isolated two pentapeptides with opiate-like activity i.e. which relaxed smooth muscle [15]. Structure analysis of these peptides yielded methionine and leucine enkephalin. The amino acid composition of Met-enkephalin is also present in (β)-lipotropin (β-LPH). This led to the discovery in 1976 of the opiate-like effects of the C-terminal peptide of β-LPH, β-endorphin by Guillemin et al. [16] and Bradbury et al. [17]. In 1977, Mains et al. found that β-LPH and ACTH are derived from the same 31 Kilo Dalton (K Da) precursor molecule [18]. This protein contains several pairs of basic amino acid residues which can yield various smaller peptides upon attack by proteolytic enzyme. In this way, ACTH and β-LPH are released

from the precursor in the anterior pituitary, and α-MSH and CLIP (corticotrophin-like intermediate lobe peptide) from ACTH in the intermediate lobe. Further biotransformation depends on the tissue enzymes and may yield α-MSH, β-endorphin, α-endorphin, γ-endorphin and smaller fragments as reported by Burbach et al. in 1980 [19]. Immunohistochemical studies revealed that the precursor molecule, to which the name pro-opiocortin (POMC) was given, is present in the brain. This material appears to originate in the nucleus arcuatus from which it is transported by peptidergic neurones to midbrain limbic structures [20].

Neuropeptide research blossomed after the discovery of the enkephalins and endorphins in the brain and the pituitary. The extrahypothalamic pathways carrying the neurohypophyseal hormones to structures were also described and these, together with many other pituitary and gut hormones present in the brain, provided a morphological basis for the central nervous system (CNS) effects of pituitary peptides as discovered a decade earlier [21,22]. If it had been known that ACTH, α-MSH and the neurohypophyseal hormones were present in the brain and that the brain peptidergic systems seem to operate independently of the pituitary, hypophysectomized rats would not have been chosen as the experimental animal for the behavioural experiments, although this was the classical endocrine approach in 1960. Nevertheless, a behavioural deficiency which seemed to depend on ACTH was found following the removal of the anterior pituitary. Avoidance learning in hypophysectomized rats could be restored with relatively small amounts of ACTH and related peptides, even though the brain contained ACTH peptides. The same is true as regards posterior lobectomy and the presence of neurohypophyseal hormones in hypothalamic and extrahypothalamic brain structures. It is of course possible that neuropeptides generated by the pituitary and the brain act in concert to mediate central nervous system (CNS) effects. However, it was known from studies in 1964 that replacement therapy in hypophysectomized rats, a combination of thyroid, gonadal and adrenocortical hormones, was nearly as effective in restoring avoidance learning as treatment with ACTH(4–10) or vasopressin [4]. It therefore seems likely that hypophysectomy is associated with a functional deficiency of neuropeptide action in the brain. This deficiency could be related to an insufficient supply of neuropeptides from the pituitary, or to metabolic disturbances that interfere with

ing endopeptidase activity (γ-EGE) was measured in the soluble fraction of various brain areas with the aid of a labelled synthetic peptide substrate (βE(15–19)). Two weeks after hypophysectomy, a significant increase in the γ-EGE activity was found in the POMC-producing cell bodies in the hypothalamus, and a significant decrease in the terminals of these cells in the septum and hippocampus. These findings suggest that hypophysectomy causes changes in the bioavailability of neuropeptides in the limbic brain. If the findings can be substantiated, the experiments in hypophysectomized rats carried out twenty years ago will prove to have been justified and the same approach might be used again today.

In any event these studies led us to the neuropeptide concept, which stresses the

CNS effects of pituitary hormones in addition to their regulating endocrine influence. The neuroactive and psychoactive effects of these hormones are dissociated from their classical function but may be just as important. The concept regards the pituitary hormones and other peptides in the brain as precursor molecules of neuropeptides. The pattern of neuropeptide generation probably is determined by the enzymatic make-up in the neuropathways and in the target tissue of these precursor molecules. A great number of peptides have now been detected in the brain. They are present in neurons and act as neurotransmitters or neuromodulators (modulating the release of neurotransmitters). In the case of pituitary hormones they serve peripheral as well as central functions, just as adrenaline acts as a hormone when released from the adrenal medulla, but as a transmitter when released from a neuron in the CNS [23]. In some cases the structural requirements for the peripheral effect of peptide hormones are the same as those needed for their central neuromodulatory action and in others they are different. For example, Tonnaer et al. have reported that the structural requirements for the central dipsogenic effect of angiotensin and related peptides are the same as those necessary for the peripheral blood pressure effects [24]. By contrast, the structural requirements for the peripheral endocrine effect of the pituitary hormones ACTH and α-MSH are different from those needed to elicit central effects [6]. Wang et al. found that in this case the pituitary hormones are precursor molecules for neuropeptides generated by proteolytic enzymes in the brain [25]. Another example is vasopressin, which is converted in the brain to highly active neuropeptides [26]. Such neuropeptides, which seem to have no apparent peripheral effects, are not formed in the posterior pituitary from which the precursor neurohypophyseal hormones are released into the blood.

Finally, β-endorphin is converted to γ-endorphin and α-endorphin related fragments which exert opposite effects in various test systems. The γ-type endorphins process neuroleptic-like activities, whereas the α-type endorphins process psychostimulant-like activities [7].

Conclusion

The study of the biotransformation of pituitary hormones in the brain and of the specific enzymes involved may be a highly fruitful line of research which could lead to the discovery of neuropeptides with specific CNS functions. It is conceivable that deficiency or abnormality in cells producing pro-opiocortin, oxytocin, vasopressin and other neuropeptides may induce disturbances in brain function, thus causing abnormal behaviour. Such deviations from normal activity may well be caused by congenital, toxic and traumatic influences or by aging or infectious diseases. If we accept the possibility that mental disturbances associated with aging may in part originate from neuroendocrine dysfunction [27], the resulting disorder may eventually be modified by treatment with neuropeptides related to ACTH and the neurohypophyseal hormones, since these neuropeptides exhibit cognitive as well as neurotrophic effects.

References

1 Smelik PG. Mechanism of hypophysial response to psychic stress. Acta Endocrinol 1960; 33: 437–443.

2 De Wied D. The significance of the antidiuretic hormone in the release mechanism of corticotropin. Endocrinology 1961; 68: 956–970.

3 De Wied D. The influence of the posterior and intermediate lobe of the pituitary and pituitary peptides on the maintenance of a conditioned avoidance response in rats. Int J Neuropharmacol 1965; 4: 157–167.

4 De Wied D. Influence of anterior pituitary on avoidance learning and escape behaviour. Am J Physiol 1964; 207: 255–259.

5 De Wied D. Effects of peptide hormones on behaviour. In: Ganong WF, Martini L, eds. London/New York: Oxford University Press, 1969; 97–140.

6 Greven HM, De Wied D. The influence of peptides derived from corticotropin (ACTH) on performance. Structure activity studies. Progr Brain Res 1973; 39: 429–442.

7 De Wied D, Jolles J. Neuropeptides derived from pro-opiocortin: behavioral, physiological and neurochemical effects. Physiol Rev 1982; 62: 976–1059.

8 De Wied D, Bohus B. Long-term and short-term effects on retention of a conditioned avoidance response in rats by treatment with long acting pitressin and α-MSH. Nature (London) 1966; 212: 1484–1486.

9 Bohus B, Gispen WH, De Wied D. Effect of lysine vasopressin and ACTH-(4–10) on conditioned avoidance behavior of hypophysectomized rats. Neuroendocrinology 1973; 11: 137–143.

10 Kozlovski GP, Nilaver G, Zimmerman EA. Distribution of neurohypophyseal hormones in the brain. Pharmacol Ther 1983; 21: 325–349.

11 Oliver C, Mical RS, Porter JC. Hypothalamic-pituitary vasculature: evidence for retrograde blood flow in the pituitary stalk. Endocrinology 1977; 101; 598–604.

12 Bergland RM, Page RB. Can the pituitary secrete directly to the brain? Endocrinology 1978; 102: 1325–1338.

13 Mezey E, Palkovits M, De Kloet ER, Verhoef J, De Wied D. Evidence for pituitary-brain transport of a behaviorally potent ACTH analog. Life Sci 1978; 22: 831–838.

14 Dorsa DM, De Kloet ER, Mezey E, De Wied D. Pituitary-brain transport of neurotensin: functional significance of retrograde transport. Endocrinology 104: 1663–1666.

15 Hughes J, Smith TW, Kosterlitz HW, Fothergill LA, Morgan BA, Morris HR. Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature (London) 1975; 258: 577–579.

16 Guillemin R, Ling N, Burgus R. Endorphines, peptides d'origine hypothalamique et neurohypophysaire à activité morphinomimétique. Isolement et structure moléculaire d'α-endorphine. CR Acad Sci (Paris) D 1976; 282: 783–785.

17 Bradbury AF, Smyth DG, Snell CR, Birdsall NJM, Hulme EC. C-fragment of lipotropin has a high affinity for brain opiate receptors. Nature (London) 1976; 260: 793–795.

18 Mains R, Eipper E, Ling N. Common precursor to corticotropins and endorphins. Proc Natl Acad Sci USA 1977; 74: 3014–3018.

19 Burbach JPH, Loeber JG, Verhoef J, Wiegant VM, De Kloet ER, De Wied D. Selective conversion of β-endorphin into peptides related to α- and γ-endorphin. Nature (London) 1980; 28: 96–97.

20 Watson SJ, Richard CW, Barchas JD. Adrenocorticotropin in rat brain: immunocytochemical localization in cells and axons. Science 1978; 200: 1180–1182.

21 Hökfelt T, Johansson O, Ljungdahl A, Lundberg JM, Schultzberg M. Peptidergic neurones. Nature (London) 284: 515–521.

22 Iversen LL. Neurotransmitters and CNS disease: introduction. Lancet 1982; II: 914–918.

23 Nemeroff ChB, Kalivas PW, Golden RN, Prange Jr AJ. Behavioral effects of hypothalamic hypophysiotropic hormones, neurotensin, substance P and other neuropeptides. Pharmacol Ther 1984; in press.

24 Tonnaer JA, Wiegant VM, De Jong W, De Wied D. Central effects of angiotensins on drinking and blood pressure: structure–activity relationships. Brain Res 1982; 236: 417–428.

25 Wang XC, Burbach JPH, Verhoef J, De Wied D. Proteolysis of adenocorticotropin in brain. J Biol Chem 1983; 258: 7942–7947.
26 Burbach JPH, Kovacs GL, De Wied D, Van Nispen JW, Greven HM. A major metabolite of arginine-vasopressin in the brain is a highly potent neuropeptide. Science 1983; 221: 1310–1312.
27 De Wied D, Van Ree JM. Neuropeptides, mental performance and aging. Life Sci 1982; 31: 709–719.