Pituitary peptides as modulators of neural functioning

M. E. A. Reith, P. Schotman, W. H. Gispen and D. de Wied

Pituitary peptides are implicated in the formation and maintenance of learned behavior. The behavioral effects are associated with concomitant changes in the metabolism of brain macromolecules.

The pituitary-adrenal system is activated by a variety of noxious stimuli ranging from body trauma to emotional stress. This system plays an important role in the adaptation of the organism to stress situations. Stress causes the release of adrenocorticotropic hormone (corticotropin or ACTH) from the anterior pituitary, which in turn activates the secretion of glucocorticosteroids from the adrenal cortex. The important role of pituitary-adrenal system hormones in adaptive behavior has been shown in many experiments, either by changing the endogenous levels of these hormones (hypophysectomy, adrenalectomy) or by administration of these hormones.

Pituitary peptides and behavior

The suggestion that the pituitary gland was involved in learning and retention of new behavior came first from observations on hypophysectomized rats; removal of the pituitary impaired the acquisition and maintenance of conditioned avoidance behavior. This behavioral abnormality can be corrected by treatment with pituitary hormones such as corticotropin, melanocyte stimulating hormone (melanotropin or MSH) or vasopressin. There is a striking dissociation between behavioral and endocrine effects since potent behavioral effects are exhibited also by fragments of vasopressin which are devoid of classical antidiuretic and pressor effects [1], as well as by fragments of corticotropin (see Fig. 1), which have no appreciable corticotropic activity [2]. In addition, many studies describe psychoactive effects of corticotropin and related peptides in intact rats in a number of behavioral situations; it turns out that these peptides enhance the retention of learned responses whether the motivation is fear, hunger or sex (for review see ref. 3).

The authors are at the Division of Molecular Neurobiology, Rudolf Magnus Institute for Pharmacology, and Laboratory of Physiological Chemistry, Medical Faculty, Institute of Molecular Biology, University of Utrecht, Utrecht, The Netherlands.

The behaviorally active core of the corticotropin molecule is located within the amino acid sequence 4–10, which is also found in α- and β-melanotropin and lipotropic hormone (lipotropin or LPH) (Fig. 1).

Thus, the pituitary gland may be a source of 'neuropeptides' which are generated from pituitary precursor molecules [2]. Structure-activity studies showed that the sequence 4–7 in the corticotropin molecule contains the essential requirements for its behavioral activity. The residue phenylalanine in position 7 seems to play a specific role and replacement of this amino acid by its D-enantiomer reverses the effect of corticotropin-like peptides on active avoidance conditioning. Peptides with D-enantiomer substitutions in other positions, on the other hand, enhance retention of conditioned responses as do all L-peptides and some of them may even have stronger effects [4].

A number of observations in rats suggest that peptides related to corticotropin affect learning by increasing the motivational value of external cues. Interestingly enough, motivational effects of corticotropin fragments have been found also in man. For example, α-melanotropin and $ACTH_{4-10}$ improved visual attention in healthy volunteers [5] and attention and motivation was increased by corticotropin in healthy volunteers subjected to a repetitious task [6]. The increase in motivation might be due to an enhancement in the state of arousal. Electrophysiological data indicate an increased arousal state in midbrain limbic structures of rats treated with $ACTH_{4-10}$ [7] and enhancement of the arousal state is also suggested by concurrent measurements of behavioral performance and heart rate responses [8].

It is conceivable that the pituitary contains a great variety of peptide sequences which are related to corticotropin or vasopressin. These may be degradation products of pituitary hormones or large precursor molecules (prohormones). Formation of new behavior then would lead to an increased production of such active substances in response to the stress involved. The peptides might enter the brain via the blood circulation, through transport along the pituitary stalk, or by release directly into the cerebrospinal fluid [4]. The last possibility is supported by the observation that intraventricular administration of antiserum against vasopressin prevents the formation of passive avoidance behavior [9]. In addition, vasopressin is much more active in active avoidance conditioning when applied intraventricularly [1].

A number of studies demonstrate that peptide hormones can actually play the role of a precursor for smaller, biologically active principles which are released enzymatically from these prohormones. For instance, H-Pro-Leu-Gly-NH_2 (melanotropin release-inhibiting factor, melanostatin) is the active factor which inhibits the release of melanotropin from the pituitary, and is released enzymatically from oxytocin by hypothalamic preparations [10,11]; the N-terminal pentapeptide of oxytocin has been reported to stimulate the release of pituitary melanotropin [12]. Furthermore, pituitary β-lipotropin appears to be the common precursor for β-melanotropin (β-lipotropin 41–58) and β-endorphin (β-lipotropin 61–91), a peptide with potent opiate activity [13]. In this context it is of interest that some behavioral effects of intracerebrally administered corticotropin cannot be demonstrated following systemic administration. For example, central injection of corticotropin induces vigorous grooming; again the N-terminal part of the corticotropin molecule is essential to produce the effect in rats [14], $ACTH_{4-7}$ being the shortest active sequence [15]. The lack of this behavioral effect following peripheral administration of corticotropin may be due to the fact that the active sequence does not reach the locus of action in sufficient quantities.

Neurochemical studies

Despite the abundance of behavioral information indicating a direct interaction between corticotropin-like peptides and the brain, molecular aspects of such interactions are sketchy. A single injection of corticotropin or β-melanotropin increased the rate of accumulation of [^{14}C]-valine into mouse brain protein by between 20 and 100%, 6 to 24 h after intraperitoneal injection of the precursor [16]. Furthermore, [^{3}H]lysine incorporation into mouse brain protein was enhanced 10–20% by $ACTH_{1-24}$ or $ACTH_{4-10}$, the incorporation being measured 10 min after subcutaneous injection of the precursor [17]. In our laboratory, rat brain proteins

```
Lipotropin41-58 (sheep)   Asp-Ser-Gly-Pro-Tyr-Lys-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp
β-Melanotropin  (pig)     Asp-Glu-Gly-Pro-Tyr-Lys-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp
α-Melanotropin                 CH3CO-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH2
ACTH1-24                            Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys
                                                                            Pro-Tyr-Val-Lys-Val-Pro-Arg-Arg
```

Fig. 1. Amino acid sequence of pituitary peptides with the behaviorally active core $ACTH_{4-10}$.

were labeled by a 5-min pulse of [^{3}H]leucine, injected directly into the diencephalon. This route of administration results in a minimal conversion of [^{3}H]leucine into ^{3}H-labeled metabolites and ^3H_2O as compared to the conversion after systemic application [18]. Under these conditions, it was found that hypophysectomy markedly decreases leucine incorporation into brain stem protein, whereas chronic treatment of hypophysectomized rats with $ACTH_{1-10}$ restored the incorporation towards that found in control rats [18].

Labeled proteins were further analyzed by sequential extraction with a hypotonic buffer, a non-ionic detergent (Triton X-100) and an ionic detergent (sodium dodecyl sulphate) resulting in one soluble and two particle-bound protein fractions. Analysis of these protein fractions on polyacrylamide gels revealed that $ACTH_{1-10}$ enhanced the incorporation of [^{3}H]leucine into all proteins. Superimposed on this general effect were minor differences in two protein bands [19]. These results suggest that the mechanism of overall protein synthesis is affected rather than specific proteins or groups of proteins.

To substantiate a direct interaction between $ACTH_{1-10}$ and central nervous structures at the molecular level, in vitro incorporation of radioactive leucine into protein was studied in brain slices. In view of its implication in the behavioral effect of corticotropin-like peptides [20], the posterior thalamus was chosen for the slice preparation. Coincubation of these slices with $ACTH_{1-10}$ resulted in an increased incorporation of [^{14}C]leucine into protein. Significant elevations of about 35% were found with concentrations between $1 \cdot 10^{-5}$ M and $5 \cdot 10^{-7}$ M of the peptide in the medium and an elevation of 13% with $1 \cdot 10^{-8}$ M [21].

Because of the differential behavioral response following application of either $ACTH_{1-10}$ or [D-Phe^7]$ACTH_{1-10}$ (see above), it was of interest to study the effect of D-enantiomer substitution on protein metabolism. Chronic treatment of hypophysectomized rats with [D-Phe^7]$ACTH_{1-10}$ led to a decreased incorporation of leucine into total brain stem protein [18], that is an effect opposite to that of [Phe^7]$ACTH_{1-10}$, while the behaviorally inert sequence $ACTH_{11-24}$ was found to be inactive. These results suggest a close correlation between the neurochemical and behavioral effects of corticotropin analogues.

More data are needed to understand how corticotropin and related peptides affect cerebral protein synthesis. The evidence obtained so far does not point to an action at the transcriptional level. Thus, chronic treatment of hypophysectomized rats with $ACTH_{1-10}$ did not alter the incorporation of uridine into rapidly labeled brain stem RNA (primarily messenger RNA). Under similar conditions no effect was found after a single injection of a high dose of $ACTH_{1-10}$, neither in intact nor in adrenalectomized rats [18]. It is therefore more likely that these corticotropin fragments act at the translational level.

Models of corticotropin action in central nervous system

By analogy with the action of corticotropin on peripheral target cells, the observed biochemical effects of corticotropin-like peptides on brain tissue may represent an intermediate step in a sequence of events leading to a functional response of the nerve cells involved. Previously it had been suggested [22] that corticotropin analogues would in fact regulate brain cellular mechanism in a manner similar to that proposed for their effects in the peripheral target cell, that is through an interaction with membrane binding sites resulting in an increased production of intracellular cyclic AMP ('second messenger') [23]. This in turn may produce many different cellular responses (changes in permeability, enzyme activity, protein synthesis, neurotransmission) via changes in protein kinase activities, depending on the effector cell involved.

These processes are illustrated in Fig. 2, together with two additional possibilities: (1) modulation of neurotransmission, for example, by interference with the release of neurotransmitter or with the interaction transmitter-postsynaptic membrane, and (2) peptidergic transmission, that is the peptide itself acts as a putative transmitter.

There are experimental data which fit such a model although many questions remain unanswered. As far as the interaction peptide-membrane receptor is concerned, we have not yet been able to obtain specific binding of radioactively labelled corticotropin fragments to brain cell membranes, however, observations made on other peptides suggest that such binding may very well exist. In addition, considerable affinity of corticotropin-like peptides for rat brain opiate receptors has been established [24]. Incubation of brain slices (taken from the posterior thalamus) with $ACTH_{1-10}$ resulted in an increase in the basal level of cyclic AMP [25] indicating that corticotropin-like peptides can affect brain metabolism through an increase in intracellular cyclic AMP. Recent evidence suggests that changes in protein kinase activities can be brought about by corticotropin: $ACTH_{1-24}$ has been found to affect the phosphorylation of synaptic plasma membrane proteins in vitro, however, these effects were not mediated by cyclic AMP [26]. It is conceivable that changes in phosphorylation of such proteins result in an altered conformation of nerve cell membranes; this in turn would change the permeability and thereby modulate neurotransmission which depends on transport of ions across the cell membrane. In addition to these permeability changes, cyclic AMP may be involved in the observed stimulation of brain protein synthesis (see above), leading to an enhanced production of enzymes involved

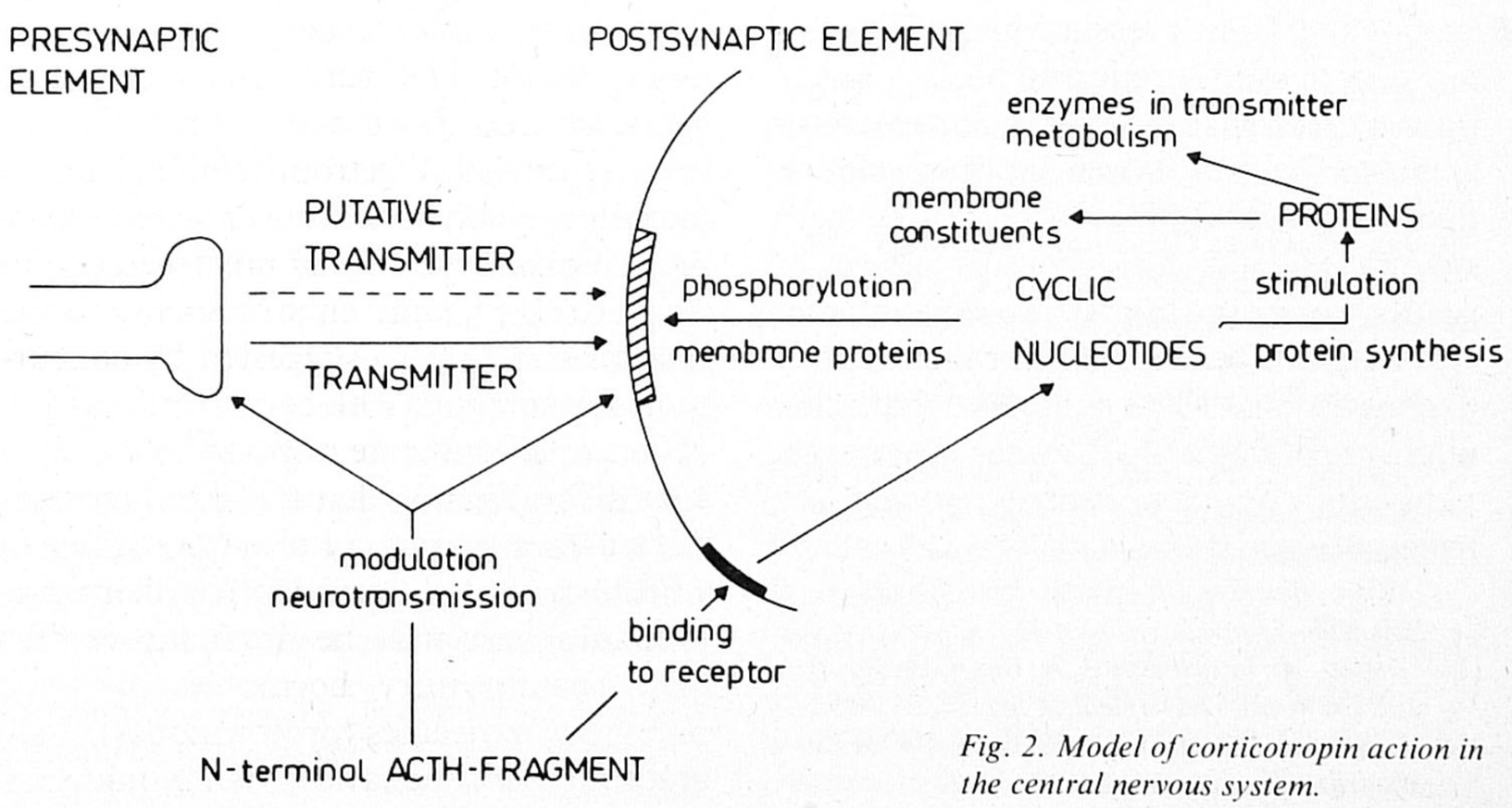

Fig. 2. Model of corticotropin action in the central nervous system.

in neurotransmitter metabolism. Indeed, corticotropin analogues have been found to change the turnover of noradrenaline in rat brain stem [27] and electrophysiological data suggest a modulatory influence of corticotropin-like peptides on transmission [28,29]. These processes, activation of cellular metabolism and resulting changes in membranes and enzymes, might produce more permanent changes in neural connectivity.

Learning of new behavior in many instances is associated with the release of corticotropin and other pituitary hormones. Increased availability of these hormones may provide the biochemical climate for enhancement of plasticity in the nervous system which allows the formation and maintenance of new behavior.

It is clear that we are only at the beginning of our understanding of how peptides regulate brain function at the molecular level. At the moment, it seems attractive to postulate that these neuropeptides act as modulators of neural functioning, thus providing the brain with an internal communication system.

References

1 De Wied, D., Bohus, B., Urban, I., Van Wimersma Greidanus, Tj. B. and Gispen, W. H. (1975) in *Peptides: Chemistry, Structure and Biology* (Walter, R. and Meienhofer, J., eds), pp. 635–643, Ann Arbor Science Publishers Inc., Ann Arbor

2 De Wied, D. (1969) in *Frontiers in Neuroendocrinology* (Ganong, W. F. and Martini, L., eds), pp. 97–140, Oxford University Press, New York

3 De Wied, D. (1974) in *The Neurosciences, Third Study Program* (Schmitt, F.O. and Worden, F.G., eds) pp. 653–666, MIT Press, Cambridge, Mass

4 De Wied, D., Witter, A. and Greven, H. M. (1975) *Biochem. Pharmacol.* 24, 1463–1468

5 Kastin, A.J., Sandman, C.A., Stratton, L.D., Schally, A.V. and Miller, L.H. (1975) in *Hormones, Homeostasis and the Brain, Progress in Brain Research* (Gispen, W. H., Van Wimersma Greidanus, Tj. B., Bohus, B. and De Wied, D., eds), Vol. 42, pp. 143–150, Elsevier, Amsterdam

6 Gaillard, A.W.K. and Sanders, A.F. (1975) *Psychopharmacologia (Berl.)* 42, 201–208

7 Urban, I. and De Wied, D. (1976) *Exp. Brain Res.* 24, 325–334

8 Bohus, B. (1975) in *Hormones, Homeostasis and the Brain, Progress in Brain Research* (Gispen, W. H., Van Wimersma Greidanus, Tj. B., Bohus, B. and De Wied, D., eds), Vol. 42, pp. 275–283, Elsevier, Amsterdam

9 Van Wimersma Greidanus, Tj. B., Dogterom, J. and De Wied, D. (1975) *Life Sci.* 16, 637–644

10 Celis, M.E., Taleisnik, S. and Walter, R. (1971) *Proc. Nat. Acad. Sci. U.S.A.* 68, 1428–1433

11 Walter, R., Griffiths, E.C. and Hooper, K.C. (1973) *Brain Res.* 60, 449–457

12 Celis, M.E., Taleisnik, S. and Walter, R. (1971) *Biochem. Biophys. Res. Commun.* 45, 564–569

13 Bradbury, A.F., Smyth, D.G. and Snell, C.R. (1976) *Biochem. Biophys. Res. Commun.* 69, 950–955

14 Gispen, W.H., Wiegant, V.M., Greven, H.M. and De Wied, D. (1975) *Life Sci.* 17, 645–652

15 Wiegant, V.M. and Gispen, W.H. (1976) *Eur. J. Pharmacol.* in press

16 Rudman, D., Scott, J.W., Del Rio, A.E., Houser, D.H. and Sheen, S. (1974) *Am. J. Physiol.* 226, 687–692

17 Dunn, A.J. and Rees, H.D. (1975) *5th International Meeting of the International Society for Neurochemistry, Barcelona, September 2–6*, (abstr.) p. 477, Seix Barral, Barcelona

18 Schotman, P., Reith, M.E.A., Van Wimersma Greidanus, Tj. B., Gispen, W.H. and De Wied, D. (1976) in *Molecular and Functional Neurobiology* (Gispen, W.H., ed.), pp. 309–344, Elsevier, Amsterdam

19 Reith, M.E.A., Schotman, P. and Gispen, W.H. (1975) *Neurobiology* 5, 355–368

20 Van Wimersma Greidanus, Tj. B., Bohus, B. and De Wied, D. (1974) *Neuroendocrinology* 14, 280–288

21 Reith, M.E.A., Schotman, P. and Gispen, W.H. (1974) *Brain Res.* 81, 571–575

22 Gispen, W.H. and Schotman, P. (1973) in *Drug Effects of Neuroendocrine Regulation, Progress in Brain Research* (Zimmerman, E., Gispen, W.H., Marks, B.H. and De Wied, D., eds), Vol. 39, pp. 443–458, Elsevier, Amsterdam

23 Sutherland, E.W. (1972) *Science* 177, 401–408

24 Terenius, L., Gispen, W.H. and De Wied, D. (1975) *Eur. J. Pharmacol.* 33, 395–399

25 Wiegant, V.M. and Gispen, W.H. (1975) *5th International Meeting of the International Society for Neurochemistry, Barcelona, September 2–6*, (abstr.) p. 205, Seix Barral, Barcelona

26 Zwiers, H., Veldhuis, H.D., Schotman, P. and Gispen, W.H. (1976) *Neurochemical Res.* in press

27 Versteeg, D.H.G. (1973) *Brain Res.* 49, 483–485

28 Urban, I., Lopes da Silva, F.H., Storm van Leeuwen, W. and De Wied, D. (1974) *Brain Res.* 69, 361–365

29 Strand, F.L. and Cayer, A. (1975) in *Hormones, Homeostasis and the Brain, Progress in Brain Research* (Gispen, W.H., Van Wimersma Greidanus, Tj. B., Bohus, B. and De Wied, D., eds) Vol. 42, pp. 187–193, Elsevier, Amsterdam

DNA-dependent DNA polymerases from eukaryotes

Erhard Wintersberger

Three distinct types of DNA polymerases have been isolated from cytoplasm and nuclei of a variety of eukaryotic cells, a fourth enzyme is obtained from mitochondria. Many properties of these enzymes are known and some ideas as to their function in the cell are beginning to emerge.

Although first reports on DNA-dependent DNA polymerases from animal cells appeared 20 years ago, it was not until recently that separation and characterization of the various enzymes was achieved and some firm ideas about their structure and function gained. One major reason for this delay in our understanding, particularly of the structure of eukaryotic DNA polymerases, is the low concentration at which these enzymes are present in the cell. It therefore turned out to be quite difficult to obtain the enzymes in a state of purity sufficient for structural characterization. Modern techniques of cell fractionation, enzyme purification, preparative gel electrophoresis and electrofocusing have helped to overcome these difficulties and some basic questions on the properties and the structure of DNA polymerases have been answered in the past few years. In addition, clues as to the role of these enzymes in the cell are emerging and will be discussed here. For a more extensive description of cellular as well as viral DNA polymerases (the latter are not dealt with in this review), the reader is referred to recent reviews [1–4].

E.W. is at the Physiologisch-Chemisches Institut der Universität, D-8700 Würzburg, Koellikerstrasse 2, G.F.R.

Four or five main classes of DNA polymerases were found in eukaryotes. Three of these enzymes are localized in nuclei and cytoplasm whereas distinct enzymes are present in organelles like the mitochondria and the chloroplasts. Many properties are shared by all the cellular DNA polymerases: they use 'activated' DNA (i.e. DNA into which single-strand breaks and gaps are introduced by incubation with small amounts of deoxyribonuclease I) as a template primer, they lack associated nuclease activity and they fail to catalyze a pyrophosphate exchange reaction, both of which are enzymatic activities associated with bacterial DNA polymerases. They differ in size, structure, primer and template specificity, necessity of SH groups for enzyme activity and in their response to mitotic stimuli. A new nomenclature for the enzymes was proposed in 1975 [3] and will be used in this review.

DNA polymerase α

The major fraction of cellular DNA polymerase activity, that of DNA polymerase α, is commonly found largely, if not