Psychoneuroendocrinology, 1977, Vol. 2, pp. 63–69. Pergamon Press. Printed in Great Britain

ACTH-LIKE PEPTIDES AND MORPHINE: INTERACTION AT THE LEVEL OF THE CNS

V. M. WIEGANT, W. H. GISPEN, L. TERENIUS* and D. DE WIED

Division of Molecular Neurobiology, Rudolf Magnus Institute for Pharmacology and Laboratory of Physiological Chemistry, Institute of Molecular Biology, University of Utrecht, Padualaan 8, Utrecht, The Netherlands

SUMMARY

(1) N-terminal fragments of ACTH antagonize morphine binding to opiate specific binding sites *in vitro*. (2) Morphine-induced analgesia in rats can be counteracted by administration (s.c.) of ACTH analogs. (3) ACTH-like peptides produce excessive grooming in rats when intraventricularly administered. (4) Excessive grooming can also be achieved by intraventricular administration of low doses of morphine or β-LPH$_{61-91}$. (5) Peptide- or morphine-induced excessive grooming can be blocked by administration (s.c.) of specific opiate antagonists (naloxone, naltrexone). (6) The structure activity requirements for ACTH-like peptides to (a) inhibit morphine binding to opiate receptors *in vitro* (b) produce excessive grooming behavior and (c) counteract morphine-induced analgesia, run fairly well parallel. (7) It is concluded that a common denominator exists in the action of ACTH-like peptides and opiates on the central nervous system.

INTRODUCTION

PITUITARY peptides have an important function in the regulation of an animal's response towards environmental stimuli. Apart from its classical endocrine effects, the pituitary hormone ACTH, and N-terminal fragments of this hormone which lack endocrine activity, are known to play a crucial role in acquisition and maintenance of a variety of behaviors in animals and man through a direct action on CNS-structures (de Wied, 1974, 1976).

Neurophysiological evidence indicates that ACTH and β-MSH counteract the morphine-induced depressant effects on spinal reflex activity *in vivo* (Zimmermann & Krivoy, 1973). ACTH$_{1-24}$ does so *in vitro* as well (Zimmermann & Krivoy, 1974) indicating a morphine–peptide interaction at the CNS level.

Peptides with opioid properties are present in brain and pituitary tissue. Peptides so far identified all turned out to be related to β-LPH. Several authors have suggested that pituitary hormones might serve as prohormones from which various peptides with distinct functions can be released by enzymatic cleavage (Celis, Taleisnik & Walter, 1971; Lazarus, Ling & Guillemin, 1976). These observations prompted us to further investigate the influence of ACTH and related peptides in different systems, in relation to morphine. Therefore experiments have been carried out to examine the effects of ACTH-like peptides *in vitro* on binding of opiates to brain cell membranes, and *in vivo* on morphine-induced analgesia. In a third series of experiments, the effect of peptides and opiate antagonists on excessive grooming in the rat was studied together with the effect of opiate-antagonists on

* Department of Medical Pharmacology, University of Uppsala, Sweden.

this peptide-induced behavior. This paper is a brief review of the present status of our work.

BRAIN OPIATE RECEPTORS

The isolation of a 'morphine-like factor' from the CSF by Terenius and Wahlström (1974) provided the first evidence for an endogenous ligand for opiate receptors which appeared to be a peptide (Terenius & Wahlström, 1975). Similar observations were reported by others (Hughes, 1975; Pasternak, Goodman & Snyder, 1975; Teschemacher, Opheim, Cox & Goldstein, 1975; Guillemin, Ling & Burgus, 1976; Bradbury, Smyth, Snell, Birdsall & Hulme, 1976). The structure of several of these peptides has now been elucidated, all of them being sequences of the pituitary hormone β-LPH (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975; Guillemin *et al.*, 1976; Bradbury *et al.*, 1976; Lazarus *et al.*, 1976).

Terenius showed that $ACTH_{1-28}$ and $ACTH_{4-10}$ have appreciable affinity for stereospecific opiate binding sites in rat brain synaptosomal plasma membrane fractions as studied by competition with DHM (Terenius, 1975). Structure–activity studies (see Table I) pointed to an active site around $ACTH_{4-10}$, with indications that a secondary site might be present in the sequence C-terminal to (4–10) (Terenius, Gispen & de Wied, 1975; Gispen,

TABLE I. INDUCTION OF EXCESSIVE GROOMING, COUNTERACTION OF MORPHINE ANALGESIA AND INHIBITION OF DIHYDROMORPHINE BINDING TO RAT BRAIN OPIATE RECEPTORS BY ANALOGS OF ACTH

Treatment	Grooming-induction potency, relative to $ACTH_{1-24}$* 0 0.5 1.0	Counteraction of morphine analgesia % inhibition† 0 50	Affinity for rat brain opiate receptors, IC_{50}
Saline			
$ACTH_{1-24}$			3×10^{-6}
$ACTH_{1-16}$-NH_2			6×10^{-6}
$ACTH_{1-10}$		n.d.‡	1.5×10^{-5}
$ACTH_{4-10}$			10^{-5}
id. 1 mg			
$ACTH_{4-7}$		n.d.	–§
$ACTH_{7-10}$		n.d.	–
$ACTH_{5-7}$		n.d.	n.d.
$ACTH_{5-14}$			3×10^{-5}
$ACTH_{5-16}$-NH_2			2×10^{-5}
$ACTH_{7-16}$-NH_2		n.d.	7×10^{-6}
$ACTH_{1-24}$			–
[D-Phe^7]$ACTH_{1-10}$		n.d.	2×10^{-5}
[D-Phe^7]$ACTH_{4-10}$			10^{-5}

* Peptides are intraventricularly injected, in doses equimolar to 3 μg $ACTH_{1-24}$
† Peptides are injected subcutaneously, in a dose of 100 μg/rat.
‡ n.d.: not determined.
§ % inhibition < 25 at 3×10^{-5}M.

Buitelaar, Wiegant, Terenius & de Wied, 1976). Substitution of [D-Phe^7] into $ACTH_{4-10}$ and $ACTH_{1-10}$ did not alter the affinity of these fragments for the DHM binding site. Interestingly, α-MSH ([acetyl-Ser^1]$ACTH_{1-13}$–NH_2), vasopressin, DG-LVP, Substance-P, MIF and TRH were inactive (Terenius, 1975; Terenius *et al.*, 1975), whereas somatostatin showed considerable activity (Terenius, 1976). Analysis of the binding characteristics of $ACTH_{1-24}$ revealed a relatively low selectivity of the peptide for agonist or antagonist. binding sites, comparable to a partial agonist–antagonist like nalorphine (Terenius, 1976). The magnitude of the affinity constants of ACTH-fragments (IC_{50}) is of the order of 10^{-5}–10^{-6} M) indicating that these peptides may not be powerful endogenous ligands for opiate receptors. Nonetheless, these data support findings by Zimmermann & Krivoy (1973) that ACTH-like peptides may interfere with morphine at the CNS level.

ANALGESIA

Morphine is a potent activator of pituitary–adrenal activity (Selye, 1936; Munson, 1973; de Wied, van Ree & de Jong, 1974). Conversely, interference by hormones of this system with the analgesic action of morphine on the CNS has been suggested (Winter & Flataker, 1951; Paroli, 1967; Gispen, van Wimersma Greidanus, Waters-Ezrin, Zimmermann, Krivoy & de Wied, 1975*a*). It was found that purified ACTH and $ACTH_{1-24}$ can antagonize the analgesic effect of morphine in the presence of the adrenal gland (Paroli, 1967; Gispen *et al.*, 1975).

Gispen *et al.* used the response to inescapable footshock as a test-system for analgesic properties as suggested by Evans (1961). Not only sensoric properties are measured under these circumstances but also CNS processes affecting the rat's motor responses (Gispen, van der Poel & van Wimersma Greidanus, 1973). Therefore in a renewed approach, the classical hot plate test according to Eddy & Leimbach (1953) was applied in order to further analyze the role of ACTH-like peptides in counteracting morphine-induced analgesia. A dose of 5 mg/kg morphine was injected i.p. 30 min prior to the hot plate trial. The peptides were administered 60 min prior to the test (100 μg/rat s.c.). Treatment with ACTH-like peptides ($ACTH_{1-24}$, $ACTH_{1-16}$, $ACTH_{4-10}$ and [D-Phe^7]$ACTH_{4-10}$) *per se* did not alter the rat's response latency (*ca.* 10 sec) on the hot plate, even if doses up to 1 mg/kg were used. Morphine given alone, increased the response latency by 12–16 sec. If, however, prior to the morphine treatment ACTH-like peptides were given, the response latency was markedly reduced (see Table I). The sequences (1–24), (1–16), (5–16), (5–14) and [D-Phe^7] $ACTH_{4-10}$ were active and reduced the response latency by approx 50% (Gispen *et al.*, 1976). The peptides (11–16), (11–17) and (11–24) were inactive. $ACTH_{4-10}$, inactive in a dose of 100 μg/rat, provoked a significant effect when given in a dose of 1 mg/rat. These data are in good agreement with the results of *in vitro* studies (Table I; Terenius, 1975; Terenius *et al.*, 1975; Gispen *et al.*, 1976) which revealed that such ACTH-like peptides have appreciable affinity for brain opiate receptors.

The results suggest that the sequence (4–10) may contain the active site. The expression of the activity, then, requires elongation of the peptide towards the C-terminus. A secondary site may provide additional affinity, without exerting intrinsic activity.

INDUCTION OF EXCESSIVE GROOMING

Intracranial and not systemic injection of ACTH or N-terminal sequences of this peptide

hormone in mammals, produces a stretching and yawning syndrome (Ferrari, Gessa & Vargiu, 1963; Gessa, Pisano, Vargiu, Crabai & Ferrari, 1967). At least in some species this syndrome is preceded by an increased display of grooming behavior (Ferrari *et al.*, 1963; Izumi, Donaldson & Barbeau, 1973; Gispen *et al.*, 1975*b*). Comparable effects were observed in pigeons (Delius, Craig & Chaudoir, 1976). In view of the short latency to the onset of the effect and its independence of endocrine activity, the induction of excessive grooming behavior is the result of a direct effect of ACTH on the CNS (Gispen *et al.*, 1975*b*). Structure-activity studies (Table I; Gispen *et al.*, 1975*b*; Wiegant & Gispen, 1977, in press) showed that the sequence (4–7) is the shortest active peptide. However, the fragments (5–14) and (5–16) are also active. This points to a secondary site beyond the 10th amino acid that provides additional activity. This secondary site may lack intrinsic activity, as may be concluded from the inactivity of the fragments (7–16) and (11–24) (see also elsewhere in this paper). In a subsequent series of experiments, the possibility of interference by specific opiate antagonists with ACTH-induced grooming was studied (Gispen & Wiegant, 1976). The injection of naloxone (1.0 mg/kg s.c.) or naltrexone (doses from 0.1 to 1.0 mg/kg s.c.) completely suppressed the grooming response induced by 1 μg $ACTH_{1-24}$ (intraventricularly). Naloxone or naltrexone treatment by itself did not provoke notable behavioral effects. These results strengthen the notion that ACTH-like peptides and opiates have a common denominator in their interaction with central nervous structures. This may find support in the data obtained in experiments on the effect of low doses of morphine on excessive grooming (see Table II). Considerable grooming behavior was induced by intraventricular injection of morphine in doses ranging from 0.05 to 0.5 μg per

TABLE II. LPH-FRAGMENTS AND MORPHINE; INDUCTION OF EXCESSIVE GROOMING

Treatment	Grooming-induction potency, relative to $ACTH_{1-24}$* (0 – 0.5 – 1.0)
Saline	
Morphine	
LPH_{61-91}	
LPH_{61-76}	
LPH_{61-69}	
LPH_{61-65}	
LPH_{65-69}	
LPH_{41-58} (β-MSH)	
LPH_{47-53} ($ACTH_{4-10}$)	
$ACTH_{1-24}$	

*Intraventricular injection occurred in doses equimolar to 3 μg $ACTH_{1-24}$, except for morphine (0.1 μg) and LPH_{61-91} (0.1 μg).

rat. This is in accordance with observations by Ayhan & Randrup (1973) who found that morphine given systemically in low doses to rats characteristically increased grooming activity. The morphine-induced excessive grooming could also be provoked in hypophysectomized animals, indicating that the effect occurs independently of the possible action of morphine on ACTH-release from the pituitary (Gispen & Wiegant, 1976). In doses higher than 0.5 μg/rat morphine depressed the overall behavior of the rat at least during the first hour after the injection. Finally, in a subsequent series of experiments, various fragments of the C-terminus of β-LPH were tested on grooming inducing activity (Table II; Gispen *et al.*, 1976 (unpublished)). Intracranial injection of LPH_{61-91}, a peptide with high affinity for brain opiate receptors and potent analgesic activity (Bradbury *et al.*, 1976), showed marked grooming activity. A subanalgesic dose (van Ree & de Wied, 1977) of 10 ng (intraventricularly) already produced a significant increase in grooming activity, indicating that the peptide is much more potent than $ACTH_{1-24}$. It should be noted, however, that in contrast to ACTH-induced grooming, administration of LPH_{61-91} in these low doses as used here, not only elicited excessive grooming but also excitation in some rats as concluded from quick movements of the body and the head, jumps, gnawing and body shakes. As was found for ACTH and morphine, LPH_{61-91} induced excessive grooming could be blocked completely by injection of naloxone (0.1 or 1 mg/kg s.c.).

Structure–activity studies were carried out to identify the amino acid sequence responsible for the induction of the behavioral response (see Table II). The sequence LPH_{61-76} was much less active than LPH_{61-91}. The shorter peptide LPH_{61-69} again was less active than LPH_{61-76}. The sequences LPH_{61-65} and $[Leu^{61}]LPH_{61-65}$ had practically no grooming activity, β-MSH, a peptide sharing the sequence LPH_{47-53} with ACTH is equipotent with $ACTH_{1-24}$ in the induction of grooming.

In conclusion, of all peptides tested so far, the LPH_{61-91} has the highest potency in inducing excessive grooming in rats. In this respect it is striking that the *in vitro* binding characteristics of this peptide suggest a mixed opiate agonist–antagonist character of the peptide (Bradbury *et al.*, 1976) like that of $ACTH_{1-24}$ (Terenius, 1976). In contrast, LPH_{61-65} has pronounced agonist properties *in vitro* (Bradbury *et al.*, 1976; Terenius, 1976), but has little if any grooming inducing activity. Obviously the potency in agonistic properties in the *in vitro* binding assay and in grooming behavior do not run parallel. These data then support the notion that opiates and β-LPH- and ACTH-analogs may have a common denominator in their neurotropic action.

DISCUSSION

Results from *in vitro* as well as *in vivo* studies cited in this paper showed that N-terminal fragments of the pituitary peptide ACTH, containing the amino acid sequence $ACTH_{4-10}$ ($= \beta\text{-}LPH_{47-53}$) interfere with morphine-binding to opiate receptors and with morphine induced analgesia.

The 'active core' of the ACTH fragments seems to be confined to the fragment (4–10), although a second site beyond the 10th amino acid may exhibit additional affinity, presumably resulting in more efficient receptor binding and higher activity in various systems.

Previously, de Wied and coworkers showed that for the direct, central effects of N-terminal ACTH fragments on acquisition and extinction of avoidance behavior,

$ACTH_{4-10}$ also comprises the essential information, although similar in opiate-receptor interaction, additional activity was found after C-terminal elongation (de Wied, Witter & Greven, 1975).

However, discrepancies exist, with respect to ACTH effects in morphine antagonism, excessive grooming and avoidance behavior: The sequences (1–10) and (4–10) are active in morphine antagonism as well as in avoidance tests but virtually inactive in producing excessive grooming. [D-Phe^7]-substitution of these peptides, potentiates the effects on morphine antagonism *in vivo*, but reverses the properties in avoidance behavior. In grooming behavior, this substitution results in activity for $ACTH_{4-10}$ and $ACTH_{1-10}$. Excessive grooming cannot be elicited by systemic injection of the peptides in contrast to morphine antagonism and the effects on learned behavior. Furthermore, there are differences with respect to structure–activity relationship for the CNS effects of ACTH fragments. It should be noted, however, that marked similarities exist between structure activity requirements in morphine antagonism and in grooming induction. The discrepancies then may, at least in part, result from the different test systems, route of administration, etc. The variety of *in vivo* effects also underscores the specificity and the complexity of the peptide–CNS interaction and suggests the existence of more than one peptide and (or) opiate sensitive site in the CNS. It is striking that LPH_{61-91} in subanalgesic doses (van Ree & de Wied, 1977) produces excessive grooming far more efficient than ACTH-like peptides, while it lacks the sequence $ACTH_{4-10}$ (β-LPH_{47-53}). On the other hand, the basic amino-acids found in the C-terminal part of both $ACTH_{1-24}$ and LPH_{61-91} could be of importance in peptide–CNS interaction.

When tested with a method for multisite analysis, $ACTH_{1-24}$ appears to be a peptide with mixed agonist–antagonist properties on the opiate receptor (Terenius, 1976). As suggested before (Gispen *et al.*, 1976) either the agonist or the antagonist properties will be dominant, depending on the test system used. Grooming could perhaps reflect the agonist properties and the counteraction of morphine analgesia, the antagonist properties. The mixed agonist–antagonist properties of β-LPH_{61-91} *in vitro* (Bradbury *et al.*, 1976) may be explained in this respect.

We thank Drs Ling and Guillemin for the donation of synthetic LPH_{61-76} and LPH-$_{61-69}$, Dr. E. C. Hulme for porcine LPH_{61-91} and Dr. H. M. Greven for synthetic LPH $_{61-65}$, LPH-$_{65-69}$, Leu-enkephalin, $ACTH_{1-24}$ and $ACTH_{4-10}$. Part of this research was supported by FUNGO-ZWO grant No. 31-13-03B.

REFERENCES

AYHAN, I. H. & RANDRUP, A. (1973) Behavioral and pharmacological studies on morphine-induced excitation of rats. Possible relation to brain catecholamines. *Psychopharmacology, Berlin*, **29,** 317–328.

BRADBURY, A. F., SMYTH, D. G., SNELL, C. R., BIRDSALL, N. J. M. & HULME, E. C. (1976) The C-fragment of lipotropin: an endogenous peptide with high affinity for brain opiate receptors. *Nature, Lond.* **260,** 793–795.

CELIS, M. E., TALEISNIK, S. & WALTER, R. (1971) Regulation of formation and proposed structure of the factor inhibiting the release of melanocyte-stimulating hormone. *Proc. natn. Acad. Sci., U.S.A.* **68,** 1428–1433.

DELIUS, J. D., CRAIG, B. & CHAUDOIR, C. (1976) Adrenocorticotropic hormone, glucose and displacement activities in pigeons. *Z. Tierpsychol.* **40,** 183–193.

EDDY, N. B. & LEIMBACH, D. (1953) Synthetic analgesics II. Dithienylbutenyl- and dithienylbutylamines. *J. Pharm. exp. Ther.* **107,** 385–393.

EVANS, W. O. (1961) A new technique for the investigation of some analgesic drugs on a reflexive behavior in the rat. *Psychopharmacologia* **2,** 318–325.

FERRARI, W., GESSA, G. L. & VARGIU, L. (1963) Behavioral effects induced by intracisternally injected ACTH and MSH. *Ann. N.Y. Acad. Sci.* **104,** 330–345.

GESSA, G. L., PISANO, M., VARGIU, L., CRABAI, F. & FERRARI, W. (1967) Stretching and yawning movements after intracerebral injections of ACTH. *Rev. Can. Biol.* **26,** 229–236.

GISPEN, W. H. & WIEGANT, V. M. (1976) Opiate antagonists suppress $ACTH_{1-24}$-induced excessive grooming in the rat. *Neurosci. Lett.* **2,** 159–164.

GISPEN, W. H., VAN DER POEL, A. & VAN WIMERSMA GRIEDANUS, TJ. B. (1973) Pituitary–adrenal influences on behaviour: responses to test situations with or without electric footshock. *Physiol. Behav.* **10,** 345–350.

GISPEN, W. H., WIEGANT, V. M., GREVEN, H. M. & DE WIED, D. (1975*b*) The induction of excessive grooming in the rat by intraventricular application of peptides derived from ACTH: structure activity studies. *Life Sci.* **17,** 645–652.

GISPEN, W. H., BUITELAAR, J., WIEGANT, V. M., TERENIUS, L. & DE WIED, D. (1976) Interaction between ACTH fragments, brain opiate receptors and morphine analgesia. *Eur. J. Pharmac.* (in press).

GISPEN, W. H., VAN WIMERSMA GREIDANUS, TJ. B., WATERS-EZRIN, C., ZIMMERMANN, E., KRIVOY, W. A. & DE WIED, D. (1975*a*) Influence of peptides on reduced response of rats to electric footshock after acute administration of morphine. *Eur. J. Pharmac.* **33,** 99–105.

GUILLEMIN, R., LING, N. & BURGUS, R. (1976) Endorphines, peptides d'origine hypothalamique et neurohypophysaire à activité morphinomimetique. Isolement et structure moleculaire d'α-endorphine. *C.r. hebd. Séanc. Acad. Sci., Paris* (Sect. D) **282,** 783–785.

HUGHES, J. (1975) Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. *Brain Res.* **88,** 295–308.

HUGHES, J., SMITH, T. W., KOSTERLITZ, H. W., FOTHERGILL, L. A., MORGAN, B. A. & MORRIS, H. R. (1975) Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature, Lond.* **258,** 557–579.

IZUMI, K., DONALDSON, J. & BARBEAU, A. (1973) Yawning and stretching in rats induced by intraventricularly administered zinc. *Life Sci.* **12,** 203–210.

LAZARUS, L. H., LING, N. & GUILLEMIN, R. (1976) β-Lipotrophin as a prohormone for the morphinomimetic peptides endorphins and enkephalins. *Proc. natn. Acad. Sci. U.S.A.* **73,** 2156–2159.

MUNSON, P. L. (1973) Effects of morphine and related drugs on the corticotrophin (ACTH)-stress reaction. In *Drug Effects on Neuroendocrine Regulation, Prog. Brain Res.* **39,** 361–372. E. Zimmermann, W. H. Gispen, B. H. Marks and D. de Wied (Eds.). Elsevier, Amsterdam.

PAROLI, E. (1967) Indagini sull'efetto antimorfinico dell'ACTH, I. Relazioni con il corticosurrene ed i livelli ematici degli 11-OH steroidi. *Arch. Ital. Sci. Farmacol.* **13,** 234–237.

PASTERNAK, G. W., GOODMAN, R. & SNYDER, S. H. (1975) An endogenous morphine-like factor in mammalian brain. *Life Sci.* **16,** 1765–1769.

SELYE, H. (1936) Thymus and adrenals in the response of the organism to injuries and intoxications. *Br. J Pathol.* **17,** 234.

TERENIUS, L. (1975) Effect of peptides and aminoacids on dihydromorphine binding to the opiate receptor. *J. Pharm. Pharmac.* **27,** 450–452.

TERENIUS, L. (1976) Somatostatin and ACTH are peptides with partial agonist-like opiate receptor selectivity. *Eur. J. Pharmac.* (in press.)

TERENIUS, L. & WAHLSTRÖM, A. (1974) Inhibitor(s) of narcotic receptor binding in brain extracts and cerebrospinal fluid. *Acta pharmac. toxicol.* **35** (*Suppl.* I), 55.

TERENIUS, L. & WAHLSTRÖM, A. (1975) Morphine-like ligand for opiate receptors in human CSF. *Life Sci.* **16,** 1759–1764.

TERENIUS, L., GISPEN, W. H. & DE WIED, D. (1975) ACTH-like peptides and opiate receptors in the rat brain: structure–activity studies. *Eur. J. Pharmac.* **33,** 395–399.

TESCHEMACHER, H., OPHEIM, K. E., COX, B. M. & GOLDSTEIN, A. (1975) A peptide-like substance from pituitary that acts like morphine. *Life Sci.* **16,** 1771–1776.

VAN REE, J. M. & DE WIED, D. (1977) Effect of neurohypophyseal hormones on morphine dependence, (Paper presented at 7th Congr. int. Soc. Psychoneuroendocrinology). *Psychoneuroendocrinology* **2** ,35–41. press).

WIED, D. DE (1974) Pituitary–adrenal system hormones and behavior. In *The Neurosciences, Third Study Program*, F. O. Schmitt and F. G. Worden (Eds.), pp. 653–666. Rockefeller University Press, New York.

WIED, D. DE (1976) Hormonal influences on motivation, learning and memory processes. *Hospital Practice* 123–131.

WIED, D. DE, VAN REE, J. M. & DE JONG, W. (1974) Narcotic analgesics and the neuroendocrine control of anterior pituitary function. In *Narcotics and the Hypothalamus*, E. Zimmermann and R. George (Eds.), pp. 251–268. Raven Press, New York.

WIED, D. DE, WITTER, A., & GREVEN H. M. (1975) Behaviourally active ACTH analogues. *Biochem. Pharmac.* **24,** 1463–1468.

WIEGANT, V. M. & GISPEN, W. H. (1977) ACTH-induced excessive grooming in the rat: latent activity of $ACTH_{4-10}$. *Behav. Biol.* (in press).

WINTER, C. A. & FLATAKER (1951) The effect of cortisone, desoxycorticosterone and adrenocorticotrophic hormone upon the responses of animals to analgesic drugs. *J. Pharm. exp. Ther.* **101,** 93–105.

ZIMMERMANN, E. & KRIVOY, W. A. (1973) Antagonism between morphine and the polypeptides ACTH, $ACTH_{1-24}$ and β-MSH in the nervous system. In *Drug Effects on Neuroendocrine Regulation, Prog. Brain Res.* **39,** pp. 383–394, E. Zimmermann, W. H. Gispen, B. H. Marks and D. de Wied (Eds.). Elsevier Amsterdam.

ZIMMERMANN, E. & KRIVOY, W. A. (1974) Depression of frog isolated spinal cord by morphine and antagonism by tetracosactin. *Proc. Soc. exp. Biol.* **146,** 575–579.