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Adrenocorticotropin/α-Melanocyte–Stimulating Hormone (ACTH/MSH)-Like Peptides Modulate Adenylate Cyclase Activity in Rat Brain Slices: Evidence for an ACTH/MSH Receptor–Coupled Mechanism

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Abstract: The regulation of adenylate cyclase activity by adrenocorticotropin/α-melanocyte–stimulating hormone (ACTH/MSH)-like peptides was investigated in rat brain slices using a superfusion method. Adenylate cyclase activity was concentration-dependently increased by ACTH-(1–24), α-MSH (EC_{50} values 16 and 6 n*M*, respectively), and [Nle^4,D-Phe^7]α-MSH (EC_{50} value 1.6 n*M*), in the presence of forskolin (1 μ*M*, optimal concentration). 1-9-Dideoxyforskolin did not augment the response of adenylate cyclase to ACTH-(1–24). Various peptide fragments were tested for their ability to enhance [^{3}H]cyclic AMP production. [Nle^4,D-Phe^7]α-MSH increased [^{3}H]cyclic AMP formation with a maximal effect of 30% and was more potent than ACTH-(1–24), ACTH-(1–16)-NH_2, α-MSH, ACTH-(1–13)-NH_2, [MetO^4]α-MSH, [MetO_2^4,D-Lys^8,Phe^9]ACTH-(4–9), ACTH-(7–16)-NH_2, ACTH-(1–10), and ACTH-(11–24), in order of potency. This structure–activity relationship resembles that found for the previously described peptide-induced display of excessive grooming. ACTH-(1–24) stimulated adenylate cyclase activity in both striatal (maximal effect, ~20%) and septal slices (maximal effect, ~40%), but not in hippocampal or cortical slices. Lesioning of the dopaminergic projections to the striatum did not result in a diminished effect of [Nle^4,D-Phe^7]α-MSH on [^{3}H]cyclic AMP accumulation, which indicates that the ACTH/MSH receptor–stimulated adenylate cyclase is not located on striatal dopaminergic terminals. ACTH-(1–24) did not affect the dopamine D_1 or D_2 receptor–mediated modulation of adenylate cyclase activity. Based on the present data, we suggest that the binding of endogenous ACTH or α-MSH to a putative ACTH/MSH receptor in certain brain regions leads to the activation of a signal transduction pathway using cyclic AMP as a second messenger. **Key Words:** Adrenocorticotropin-(1–24)—α-Melanocyte–stimulating hormone—Melanocyte-stimulating hormone receptor—Adenylate cyclase activity—Forskolin—Rats. **Florijn W. J. et al.** Adrenocorticotropin/α-melanocyte–stimulating hormone (ACTH/MSH)-like peptides modulate adenylate cyclase activity in rat brain slices: Evidence for an ACTH/MSH receptor–coupled mechanism. *J. Neurochem.* **60,** 2204–2211 (1993).

Numerous studies have shown that administration of adrenocorticotropin/α-melanocyte–stimulating hormone (ACTH/MSH)-like peptides to intact animals influences performance in a number of behavioral paradigms (reviews: Sandman and O'Halloran, 1986; De Wied and Wolterink, 1988; Eberle, 1988). Immunocytochemical studies have shown a widespread occurrence of ACTH/MSH-like peptides in the brain (Jacobowitz and O'Donohue, 1978; Watson and Akil, 1979; Palkovits et al., 1987). The exact brain sites and signal pathways that are responsible for the effects of these neuropeptides on behavior are largely unknown. However, the induction of excessive grooming by the intracerebroventricular administration of ACTH/MSH-like peptides has been extensively studied with respect to both behavioral and biochemical aspects (reviews: Gispen and Isaacson, 1986; Wiegant et al., 1986). A structure–activity rela-

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Abbreviations used: ACTH/MSH, adrenocorticotropin/α-melanocyte–stimulating hormone; DOPAC, 3,4-dihydroxyphenylacetic acid; IBMX, 3-isobutyl-1-methylxanthine; MANOVA, analysis of variance for repeated measures; 6-OHDA, 6-hydroxydopamine; NPA, *N*-*n*-propylnorapomorphine; PKC, protein kinase C.

tionship study performed with a variety of ACTH/MSH fragments indicated that a MSH receptor–mediated mechanism may underlie the display of excessive grooming; a close resemblance between the structure–activity relationship of α-MSH fragments and analogues on the expression of excessive grooming in the rat and on the pigment dispersion in *Xenopus* melanophores was observed (Spruijt et al., 1985). Furthermore, it has been reported that specific melanotropin binding sites are distributed widely within the rat brain (Tatro, 1990). The possible existence of a parallel between the peripheral and central mechanism of action of ACTH was the subject of investigations more than a decade ago by Wiegant et al. (1979). Using striatal slices, these investigators found a stimulatory effect of micromolar concentrations of ACTH-(1–24) on adenylate cyclase in striatal slices. Moreover, ACTH-(1–24) and α-MSH have been reported to stimulate adenylate cyclase in primary astroglial cell cultures from newborn mice and rats (Van Calker et al., 1983; Zohar and Salomon, 1992) and similar results were obtained with primary cultures of mouse cortical or striatal neurons (Weiss et al., 1985). In the latter study, the intrinsic activity of ACTH-(1–24) and α-MSH was enhanced by forskolin.

In the present report, we describe detailed structure–activity studies that were performed using various ACTH/MSH-related peptides to characterize the active site in the ACTH sequence responsible for the enhancement of adenylate cyclase activity in striatal slices. Furthermore, the effect of ACTH-(1–24) on adenylate cyclase activity was examined in different brain regions to explore possible regional selectivity. We also addressed the possible involvement of the dopaminergic system on ACTH-induced [^{3}H]cyclic AMP formation. A combination of a superfusion method and a very sensitive conversion method for the measurement of adenylate cyclase activity, that has been used previously to study the receptor/effector system beyond opioid and dopamine receptors (Schoffelmeer et al., 1985; De Vries et al., 1990), was used in this study.

MATERIALS AND METHODS

Preparation and superfusion of rat brain slices

Superfusion of rat brain slices and determination of adenylate cyclase activity were performed as described previously (De Vries et al., 1990). In brief, male Wistar rats, weighing 140–200 g, were killed by decapitation and their brains were excised rapidly and stored in ice-cold buffer. All experimental protocols involving laboratory animals that were used to obtain the results described in this study were approved by both the institutional review committees of the Medical Faculty of the Utrecht University and that of the Medical Faculty of the Free University of Amsterdam and met the guidelines of the governmental agency.

Subsequently, the brain structures were dissected and cut into 0.3 × 0.3 × 0.2-mm slices using a McIlwain tissue chopper. The slices were then transferred to a beaker containing Krebs–Ringer bicarbonate buffer. The composition of the medium was as follows (m*M*): NaCl 121, KCl 1.87, KH_2PO_4 1.17, $MgSO_4$ 1.17, $CaCl_2$ 1.22, $NaHCO_3$ 20, and glucose 11.1, pH 7.4, continuously gassed with 95% O_2/5% CO_2 at 37°C. The slices were washed twice with 5 ml of Krebs–Ringer bicarbonate medium containing 1 m*M* 3-isobutyl-1-methylxanthine (IBMX). The slices were then incubated for 1 h with buffer containing 10 μCi/2.5 ml of [^{3}H]-adenine under a 95% O_2/5% CO_2 atmosphere at 37°C in a shaking water bath. Every 15 min, the buffer containing 10 μCi/2.5 ml of [^{3}H]adenine was refreshed. Subsequently, ~20 mg of the labeled slices were transferred to each of 24 superfusion chambers (volume, 0.2 ml) and the slices were superfused at a rate of 0.1 ml/min with carbogenated medium containing IBMX, to prevent breakdown of [^{3}H]-cyclic AMP by phosphodiesterase, at 37°C. After 40 min of superfusion, the slices were exposed for 10 min to either the peptides or vehicle in the presence or absence of forskolin. In experiments with dopamine receptor agonists and antagonists, exposure of the slices with peptides was prolonged to 20 min, whereas dopamine, quinpirole, or SKF 38393 was added at the start of the second half of this period. The slices were then superfused with 5% cold trichloroacetic acid containing 1 m*M* nontritiated ATP and cyclic AMP for 15 min, to extract the tritiated nucleotides. The effluent was used for the chromatographical separation of [^{3}H]cyclic AMP from other ^{3}H-nucleotides, as previously described by Salomon et al. (1974). In general, only a small part of [^{3}H]cyclic AMP was lost by enzymatic degradation, egress from slices, or during the extraction procedure. The activity of adenylate cyclase was expressed as % of conversion = ([^{3}H]cyclic AMP/^{3}H-nucleotides) × 100.

Induction of the 6-hydroxydopamine (6-OHDA) lesion

Two hours before the time of surgery desipramine (25 mg · kg^{-1}) was injected intraperitonally. The rats were anesthetized with Hypnorm (0.7 ml · kg^{-1}, intramuscularly) and placed in a stereotaxic apparatus. Subsequently, 6-OHDA (6 μg/2 μl dissolved in saline containing 0.02% ascorbic acid) was injected unilaterally in the left medial forebrain bundle (stereotaxic coordinates with bregma as overall zero were posterior −4.4, lateral 1.1, and ventral 7.8 mm) and in the ventral tegmental area (stereotaxic coordinates with bregma as overall zero were posterior −4.0, lateral 0.8, and ventral 8.0 mm) of nine rats. Three to 5 weeks after the lesion, the animals were killed and the striata were dissected. A small part of the left or right striatum was stored at −80°C and was used for the measurement of dopamine and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC). The remaining parts of the left and right striata were processed as described above.

HPLC analysis

Striatal tissue of the control and lesioned striata were homogenized in 240 μl of 0.1 *M* perchloric acid and then centrifuged at 3,000 *g* for 10 min at 4°C. The supernatant was transferred to sample vials containing 20 μl of 0.5 *M* sodium acetate to raise the pH of the solution to ~3.5 (final volume, 240 μl). The samples were stored at −80°C before analysis by HPLC using a Hewlett-Packard series 1081B chromatograph equipped with an Antec Leyden electrochemical detector (Leiden, The Netherlands) for their content of dopamine and its metabolite, DOPAC. A SUPELCO LC-8-DB column with 250 × 4.6-mm internal diameter

and particle diameter of ~5 μm was used for the separation. The mobile phase consisted of a 0.1 *M* Na_3PO_4 buffer, pH 3.5, containing 60 ml of MeOH, 0.25 mmol/L of Na_2EDTA, and 2.9 mmol/L of octanesulfonic acid. The oxidation of the detector potential was set at 650 mV with respect to an Ag/AgCl reference electrode.

Drugs

[^{3}H]Adenine (25 Ci/mmol) was purchased from Amersham; IBMX, dopamine hydrochloride, forskolin, cyclic AMP from Sigma (St. Louis, MO, U.S.A.); ATP from Boehringer Mannheim (Mannheim, F.R.G.); SKF 38393 was from Smith, Kline and French (Philadelphia, PA, U.S.A.); quinpirole (LY 171555) was a gift from Eli Lilly (Indianapolis, IN, U.S.A.), [Nle^4,D-Phe^7]α-MSH was from Bachem AG (Bubendorf, Switzerland); and all other ACTH/MSH-related peptides were a gift from Dr. J. Van Nispen, Organon International BV, Oss, The Netherlands.

Statistics

Statistical analysis of the data was performed by one-way ANOVA, followed by Tukey *B* tests in the case of equal or nearly equal sample sizes, or by Student–Newman–Keuls tests in the case of unequal sample sizes. A p value of <0.05 was considered to indicate a significant difference. Analysis of variance for repeated measurements (MANOVA) was used to assess statistical difference between dose–response curves.

RESULTS

Forskolin augments the response of adenylate cyclase to ACTH-(1–24)

Usually, basal adenylate cyclase activity, expressed as the percentage of total tissue ^{3}H-nucleotide content that is converted into [^{3}H]cyclic AMP, comprised 0.27 ± 0.01% (n = 120). Upon exposure of striatal slices to 1 μ*M* ACTH-(1–24), the formation of [^{3}H]-cyclic AMP was stimulated by 20–30% in some, but not in all, pilot experiments. However, after the addition of the adenylate cyclase activator forskolin (1 μ*M*), which itself increased adenylate cyclase activity almost threefold, a consistent stimulatory effect of ACTH-(1–24) on adenylate cyclase activity of ~20% was observed. In contrast, 1 μ*M* 1-9-dideoxyforskolin did not stimulate adenylate cyclase by itself, nor did it potentiate the response of adenylate cyclase to ACTH-(1–24). ACTH-(1–24) by itself caused a small, but statistically insignificant increase (Table 1).

The magnitude of the stimulation of adenylate cyclase by ACTH-(1–24) depended on the concentration of forskolin used, optimal concentrations being in the range of 0.3–1.0 μ*M* forskolin (Fig. 1). Based on these data, a forskolin concentration of 1 μ*M* was chosen for all further experiments.

Structural requirements for the modulation of adenylate cyclase activity

ACTH-(1–24), α-MSH, and the selective α-MSH–receptor ligand, [Nle^4,D-Phe^7]α-MSH, concentration-dependently increased the formation of [^{3}H]cyclic AMP in the presence of 1 μ*M* forskolin (Fig. 2).

TABLE 1. *Forskolin, but not its analogue 1-9-dideoxyforskolin, potentiates the effect of ACTH-(1–24) on the activity of adenylate cyclase in striatal slices*

Drugs present in the superfusion medium	Adenylate cyclase activity (% conversion of [^{3}H]ATP into [^{3}H]cyclic AMP)	
	Without ACTH-(1–24)	With ACTH-(1–24)
None	0.22 ± 0.01	0.27 ± 0.02
Forskolin (1 μ*M*)	0.62 ± 0.02	0.79 ± 0.02[a]
1-9-Dideoxyforskolin (1 μ*M*)	0.22 ± 0.01	0.26 ± 0.02

Each value is the mean ± SEM of 12 observations obtained in six separate experiments.

[a] Significant difference ($p < 0.05$) with forskolin-affected adenylate cyclase activity (ANOVA, followed by Tukey *B* tests).

ACTH-(1–24) and α-MSH were almost equipotent (EC_{50} values of 16 and 6 n*M*, respectively), whereas [Nle^4,D-Phe^7]α-MSH was slightly more potent (EC_{50} value of 1 n*M*) in stimulating [^{3}H]cyclic AMP formation. The intrinsic activity of [Nle^4,D-Phe^7]α-MSH was higher compared with that of ACTH-(1–24) and α-MSH.

Other structurally related peptides were tested in the adenylate cyclase assay. The results are summarized in Table 2. Desacetyl α-MSH [ACTH-(1–13)-NH_2] appeared to be the shortest peptide fully active in the adenylate cyclase assay.

Regional specificity and localization of the site of action

The magnitude of the effect of ACTH-(1–24) on adenylate cyclase activity depends on the brain area used. The largest increase in [^{3}H]cyclic AMP production was found in septal slices at 1 and 10 μ*M* ACTH-(1–24) (Fig. 3), the response being twice that found in striatal slices. In neocortical and hippocampal slices, adenylate cyclase was not activated by ACTH-(1–24) (Table 3).

Destruction of dopaminergic projections to the left striatum, after unilateral injection of the neurotoxin 6-OHDA in the medial forebrain bundle and in the ventral tegmental area, resulted in the nearly total absence of dopamine and its major metabolite, DOPAC. Dopamine levels were 34.49 ± 6.93 and 0.19 ± 0.42 ng/mg of protein in the control and lesioned side, respectively. DOPAC levels were 45.85 ± 6.07 and 0.49 ± 0.17 ng/mg of protein in the control and lesioned side, respectively. The effect of [Nle^4,D-Phe^7]α-MSH on adenylate cyclase, however, was the same in the lesioned and control part of the striatum and also compared with that of control rats (Fig. 4).

No effect of ACTH-(1–24) on dopamine receptor–mediated [^{3}H]cyclic AMP production

Stimulation of dopamine D_2 receptors with the dopamine D_2 receptor agonist quinpirole partly antagonized the forskolin- or dopamine D_1 receptor–stimulated [^{3}H]cyclic AMP production. Blockade of dopa-

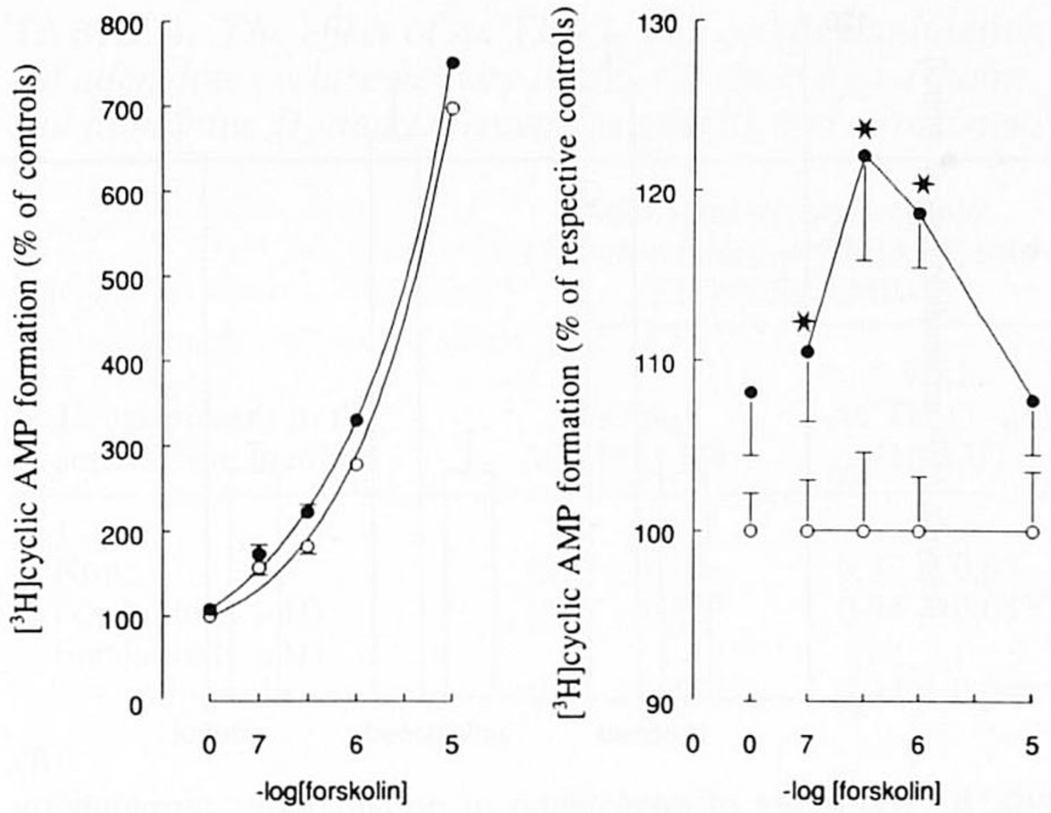

FIG. 1. The effect of increasing concentrations of forskolin on adenylate cyclase activity in the absence (○) and presence (●) of ACTH-(1–24). **Left:** Data expressed as a percentage of basal [^{3}H]-cyclic AMP formation. **Right:** Data obtained in the presence of 1 μM ACTH-(1–24) expressed as a percentage of their respective controls. Each value is the mean ± SEM of 12–19 observations obtained in seven separate experiments. *Differences are significant at forskolin concentrations of 10^{-7}, 3×10^{-7}, and 10^{-6} M ($p < 0.05$, ANOVA, followed by Student–Newman–Keuls tests). MANOVA revealed a significant difference between the concentration–response curves of the left panel ($p < 0.05$).

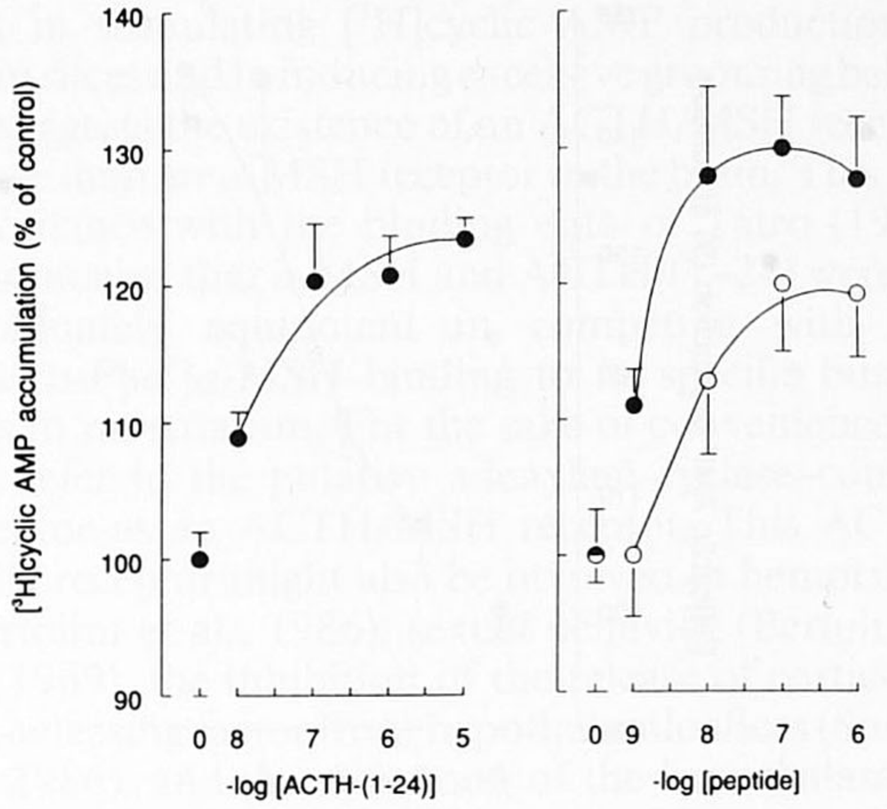

FIG. 2. Concentration–response curves for the effect of ACTH-(1–24), shown in the **left** panel, and α-MSH (○) and [Nle^4,D-Phe^7]α-MSH (●), shown in the **right** panel, on the forskolin-stimulated [^{3}H]cyclic AMP production in rat striatal slices. Data are expressed as a percentage of controls (1 μM forskolin added to the medium; means ± SEM). Each value is the mean of 10–12 observations obtained in nine separate experiments. As a positive control, ACTH-(1–24) was tested simultaneously with the various α-MSH concentrations (the effect on adenylate cyclase activity comprised 115.4 ± 3.2% of control). α-MSH was used as a positive control for the [Nle^4,D-Phe^7]α-MSH concentration–response curve (the effect on adenylate cyclase activity comprised 123.1 ± 6.4% of control). MANOVA revealed a significant difference between the α-MSH and the [Nle^4,D-Phe^7]α-MSH concentration–response curves.

mine D_2 receptors by sulpiride resulted in an increased formation of [^{3}H]cyclic AMP in striatal slices exposed to 40 μM dopamine. The addition of 10 μM ACTH-(1–24) did not affect any of the forskolin- or dopamine D_1 or D_2 receptor–induced changes in adenylate cyclase activity (Table 4).

DISCUSSION

Ever since it was recognized that ACTH/MSH-like peptides may modulate brain function, the question of their molecular mechanism of action in the brain has been addressed. Numerous reports described effects of ACTH or α-MSH on the receptor-mediated activation of adenylate cyclase of peripheral target tissues like adrenal glomerulosa cells, melanoma, and melanocytes (for a review: Eberle, 1988; Gallo-Payet and Payet, 1989), whereas observations on brain tissue are few. Most investigators reported that ACTH did not affect adenylate cyclase activity in the brain, but Wiegant et al. (1979) observed a small stimulatory effect of ACTH-(1–24) in striatal slices in vitro. The small and variable effect of ACTH-(1–24) in striatal slices frustrated the further characterization of the site of action. The use of a very sensitive adenylate cyclase assay in combination with a superfusion method (Schoffelmeer et al., 1985; De Vries et al., 1990), and the availability of the adenylate cyclase activator forskolin, enabled us to examine the possibility of the existence of an ACTH and/or α-MSH receptor–coupled adenylate cyclase system in the rat brain.

Pilot experiments had shown the benefits of using forskolin for the measurement of the modulation of adenylate cyclase activity by ACTH-(1–24). Forskolin significantly stimulated [^{3}H]cyclic AMP accumulation at the lowest concentration tested (100 nM).

TABLE 2. *Influence of various peptide fragments on the production of [^{3}H]cyclic AMP from striatal slices*

Peptide fragment	[^{3}H]Cyclic AMP production (% of control)
ACTH-(1–24)	118.1 ± 3.2[a]
ACTH-(1–10) + ACTH-(11–24)	106.5 ± 2.6
ACTH-(1–16)-NH_2	124.9 ± 4.4[a]
ACTH-(1–13)-NH_2	116.0 ± 2.9[a]
α-MSH	120.1 ± 2.4[a]
[MetO^4]α-MSH	108.8 ± 2.0
[Nle^4,D-Phe^7]α-MSH	129.8 ± 3.3[a]
ACTH-(7–16)-NH_2	107.9 ± 2.2
[MetO$_2^4$,D-Lys^8,Phe^9]ACTH-(4–9)[b]	108.4 ± 5.0

The effect on [^{3}H]cyclic AMP production of various peptide fragments (peptide concentration 0.1 μM) was tested in the presence of 1 μM forskolin for 10 min. Each value represents the mean ± SEM of 12 observations obtained in three separate experiments per group of three to five fragments, each group with its respective α-MSH control.

[a] Significant difference ($p < 0.05$) with the respective positive control value (ANOVA, followed by Tukey B tests).

[b] Tested at a concentration of 1 μM.

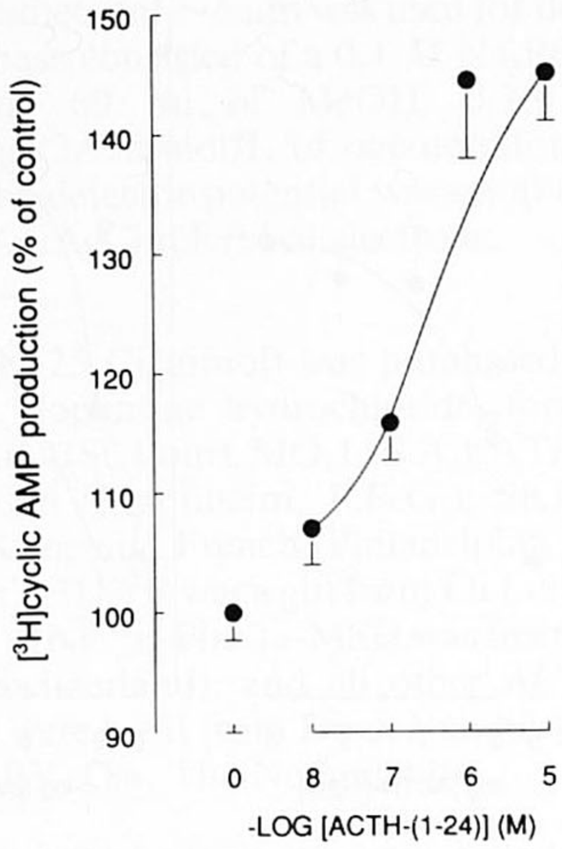

FIG. 3. Effect of increasing concentrations of ACTH-(1–24) on forskolin-stimulated [^{3}H]cyclic AMP accumulation in septal (●) slices. Data are expressed as a percentage of controls (1 μM forskolin added to the medium; means ± SEM). Each value is the mean of 12 observations obtained in three separate experiments.

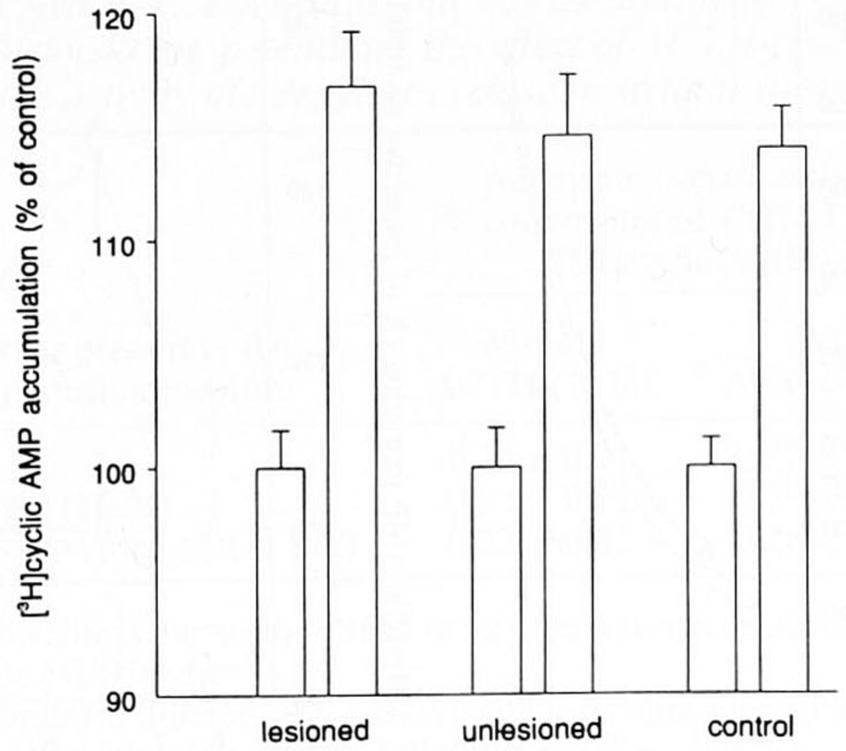

FIG. 4. The effect of destruction of dopaminergic terminals by 6-OHDA injection in the median forebrain bundle and ventral tegmental area on [Nle^4,D-Phe^7]α-MSH–stimulated adenylate cyclase activity in striatal slices. After pretreatment with desipramine (25 mg · kg^{-1}, i.p.), 6-OHDA was injected unilaterally in the left median forebrain bundle and in the left ventral tegmental area of nine rats. Three to 5 weeks after the lesion, slices of the lesioned left striatum and the nonlesioned right striatum were prepared. [Nle^4,D-Phe^7]α-MSH–stimulated [^{3}H]cyclic AMP production was measured in four separate experiments (n = 16). The data were expressed as a percentage of controls (1 μM forskolin added to the medium; means ± SEM). Absolute control values are 0.70 ± 0.02, 0.84 ± 0.03, and 0.78 ± 0.03% of conversion, respectively, for the slices obtained from the lesioned, nonlesioned, and control striata.

The use of forskolin in this study is not based on its low-affinity reversible binding to the catalytic subunit, resulting in a direct activation of adenylate cyclase, but rather on the ability of the compound to potentiate the effect of receptor-mediated [^{3}H]cyclic AMP generation, possibly by stabilizing the complex between the GTP-binding regulatory protein and the catalytic unit of adenylate cyclase via reversible binding to a high-affinity site (Bouhelal et al., 1985; Nelson and Seamon, 1986). That the adenylate cyclase binding properties of forskolin are important for the potentiation of the effect of ACTH-(1–24) on adenylate cyclase activity was illustrated by using 1-9-dideoxyforskolin, a forskolin analogue that lacks forskolin's effect on adenylate cyclase, but shares all other reported effects of forskolin such as the inhibition of muscimol-stimulated Cl^- flux through the $GABA_A$ receptor/Cl^- channel complex and the modulation of voltage-dependent K^+ channels (Laurenza et al., 1989); in the presence of 1-9-dideoxyforskolin, ACTH-(1–24) did not significantly affect adenylate cyclase activity (Table 1). The response pattern of adenylate cyclase to ACTH-(1–24) in the presence of different concentrations of forskolin (Fig. 1) was essentially the same as that reported by Daly et al. (1982) for dopamine. Their report showed a bell-shaped curve with a maximal effect at 100 nM forskolin; we observed a bell-shaped curve with a relative maximal effect at 300 nM forskolin (Fig. 1, right panel). The absolute difference (in dpm or percentage of conversion), however, was maximal at a dose of 1 μM forskolin. Hence, this concentration was taken for further experiments.

TABLE 3. *Regional differences in the effect of 1 μM ACTH-(1–24) on adenylate cyclase activity*

Brain structure	Adenylate cyclase activity (% conversion of [^{3}H]ATP into [^{3}H]cyclic AMP) Without ACTH-(1–24)	With ACTH-(1–24)
Striatum	0.68 ± 0.02	0.81 ± 0.03[a]
Septum	0.66 ± 0.04	0.97 ± 0.09[a]
Hippocampus	0.39 ± 0.02	0.44 ± 0.02
Cortex	0.68 ± 0.03	0.76 ± 0.04

Forskolin 1 μM was present in the medium. Each value is the mean ± SEM of 12 observations obtained in six separate experiments. In the absence of forskolin, percentages of [^{3}H]cyclic AMP formation were 0.27 ± 0.01, 0.19 ± 0.02, 0.18 ± 0.01, and 0.32 ± 0.02 for striatal, septal, hippocampal, and cortical slices, respectively.

[a] $p < 0.05$, for difference with forskolin-affected adenylate cyclase activity without ACTH-(1–24) (ANOVA, followed by Tukey B tests).

The EC_{50} values obtained from the ACTH-(1–24) and α-MSH concentration–response curves were ~16 and ~6 nM, respectively, and are in the same range as those reported for the effects of the peptides in primary neuronal cortical cultures [EC_{50} value of 10 nM for ACTH-(1–24), 100 nM for α-MSH; Weiss et al., 1985], primary astroglia cultures [EC_{50} value of 10 nM for ACTH-(1–24), 30 nM for α-MSH; Van Calker et al., 1983], melanoma [EC_{50} value of 200 nM for α-MSH, 1 μM for ACTH-(1–24); Eberle, 1988], and adrenal glomerulosa cell cultures [EC_{50} value of

TABLE 4. *The effect of ACTH-(1–24)) on the modulation of adenylate cyclase activity in striatal slices by forskolin and dopamine D_1 and D_2 receptor agonists and antagonists*

Drugs present in the superfusion medium	Adenylate cyclase activity (% conversion of [^{3}H]ATP into [^{3}H]cyclic AMP)	
	Without ACTH-(1–24)	With ACTH-(1–24) (10 μM)
(A)		
None	0.33 ± 0.01	0.37 ± 0.01
Forskolin (1 μM)	0.73 ± 0.02[a]	0.94 ± 0.04[a,b]
Forskolin (1 μM) + quinpirole	0.59 ± 0.03[a,b]	0.74 ± 0.04[a–c]
(B)		
None	0.22 ± 0.01	0.23 ± 0.01
Dopamine (40 μM)	0.37 ± 0.01[a]	0.38 ± 0.02[a]
Dopamine (40 μM) + sulpiride (1 μM)	0.55 ± 0.02[a,d]	0.58 ± 0.03[a,d]
(C)		
SKF 38393 (10 μM)	0.63 ± 0.02	0.61 ± 0.02
SKF 38393 (10 μM) + quinpirole (0.1 μM)	0.52 ± 0.03[e]	0.50 ± 0.01[e]
SKF 38393 (10 μM) + quinpirole (1 μM)	0.48 ± 0.01[e]	0.49 ± 0.02[e]

ACTH-(1–24) was added 10 min before all other compounds. The activity of adenylate cyclase is expressed as the percentage of conversion of ^{3}H-nucleotide precursors to [^{3}H]cyclic AMP.

[a] $p < 0.05$, for difference with controls.

[b] $p < 0.05$, for difference with forskolin.

[c] $p < 0.05$, for difference with forskolin + ACTH-(1–24).

[d] $p < 0.05$, for difference with dopamine.

[e] $p < 0.05$, for difference with SKF 38393 (ANOVA followed by Tukey *B* tests).

10 nM for ACTH-(1–24); Gallo-Payet and Payet, 1989].

The present data support the notion that the adenylate cyclase activity–stimulating effect involves activation of a putative MSH-like receptor. First, in the adenylate cyclase assay, [Nle^4,D-Phe^7]α-MSH was more potent and had a higher intrinsic activity than α-MSH or ACTH-(1–24) (Fig. 2, right panel). This MSH analogue is very potent in peripheral MSH receptor (bio)assays (Sawyer et al., 1980), but inactive in adrenal ACTH receptor assays (Baumann et al., 1986). Second, the structure–activity relationship found in the adenylate cyclase assay (Table 2) is very similar to that found for the induction of excessive grooming (Fig. 5). Spruijt et al. (1985) compared the structure–activity relationship found for peptide-induced grooming behavior in the rat and pigment dispersion in *Xenopus* melanophores and observed a strong resemblance. These investigators therefore suggested the existence of a putative MSH receptor in the brain. Hirsch et al. (1984) and Al-Obeidi et al. (1989) reported that central and peripheral α-MSH receptors require some similar, but also some different, recognition characteristics for structural and conformational properties of α-MSH analogues. That ACTH-(1–24), ACTH-(1–16)-NH_2, and α-MSH are almost equipotent in stimulating [^{3}H]cyclic AMP production in brain slices and in inducing excessive grooming behavior suggests the existence of an ACTH/MSH receptor rather than an α-MSH receptor in the brain. This is in accordance with the binding data of Tatro (1990), who showed that α-MSH and ACTH-(1–24) were approximately equipotent in competing with ^{125}I-[Nle^4,D-Phe^7]α-MSH binding to its specific binding sites in rat striatum. For the sake of convenience, we will refer to the putative adenylate cyclase–coupled receptor as an ACTH/MSH receptor. This ACTH/MSH receptor might also be involved in hemorrhage (Bertolini et al., 1986), sexual behavior (Bertolini et al., 1969), the inhibition of the release of corticotropin-releasing factor from hypothalamic slices (Suda et al., 1986), and the activation of the hypothalamopituitary axis after central application of these peptides (Wiegant et al., 1979, and V. M. Wiegant, personal communication). An ACTH/MSH receptor may occur in peripheral neurons as well because ACTH-(1–24), ACTH-(1–16)-NH_2, ACTH-(1–13)-NH_2, and α-MSH are equipotent in the facilitation of peripheral nerve repair (Van der Zee et al., 1988). In lacrimal gland cells, binding of ^{125}I-[Nle^4,D-Phe^7]α-MSH to its receptor could be displaced by ACTH-(1–24), ACTH-(1–13)-NH_2, and α-MSH (Entwistle et al., 1990; Leiba et al., 1990). These findings are in line with an early study of Jahn et al. (1982), who reported that ACTH-(1–24) and α-MSH were equipotent in the stimulation of adenylate cyclase activity in the lacrimal gland.

Desacetyl α-MSH was nearly as active as α-MSH in the adenylate cyclase assay and in the receptor binding assay (Tatro, 1993), showing that acetylation of desacetyl α-MSH is not a prerequisite for receptor binding and activation of adenylate cyclase. This was also observed by Eberle (1988) for the stimulation of

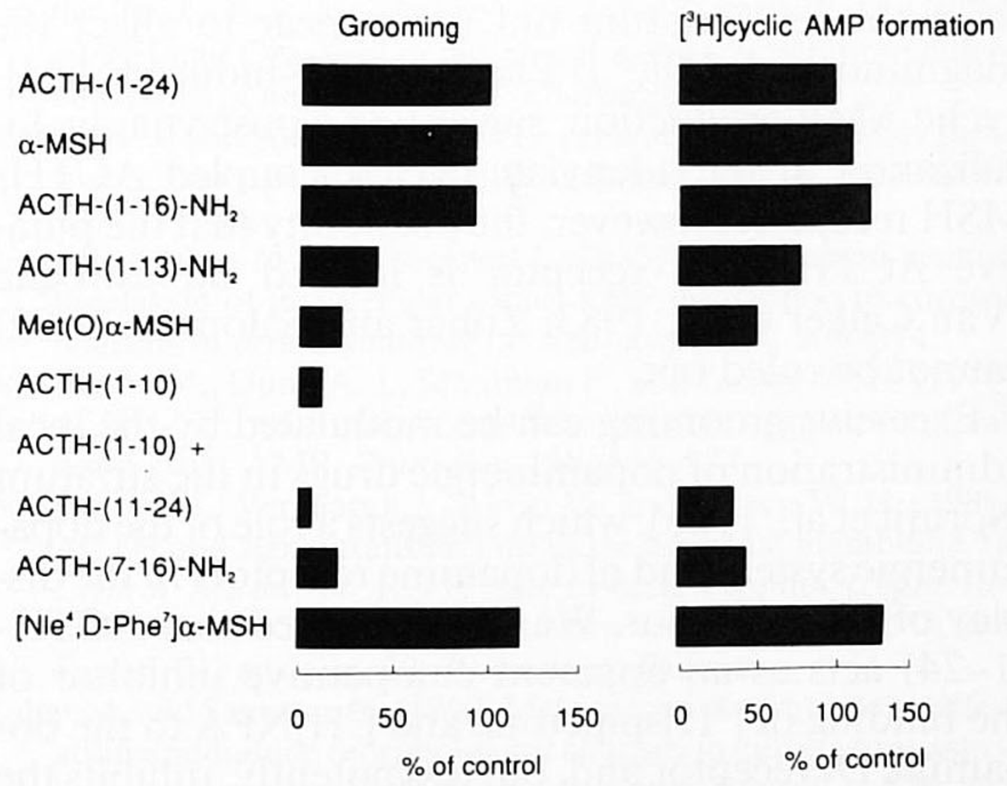

FIG. 5. Structure–activity relationship between peptide-induced grooming in vivo and [^{3}H]cyclic AMP formation in striatal slices in vitro. The effect of ACTH-(1–24) was taken as 100% (=control). [Data for the construction of the structure–activity profile of peptide-induced grooming were obtained from Gispen et al. (1975) and Spruijt et al. (1986).]

pigment dispersion in *Xenopus* melanophores. Gispen et al. (1975), however, showed that desacetyl α-MSH was less potent than α-MSH in inducing grooming behavior, although a nearly full response could be obtained with a higher dosage.

ACTH-(7–16)-NH_2, which has been shown to be even more potent than ACTH-(1–24) in displacing the binding of [^{3}H]*N*-*n*-propylnorapomorphine ([^{3}H]-NPA) to the dopamine D_2 receptor (Florijn et al., 1991), appeared to be virtually inactive in the adenylate cyclase assay, indicating that the dopamine D_2 receptor is not involved in the stimulatory effect of α-MSH on [^{3}H]cyclic AMP formation (see also below).

Org 2766, i.e., [MetO_2^4,D-Lys^8,Phe^9]ACTH-(4–9), has been reported to facilitate adaptive behavior and memory processes (for review: De Wied and Wolterink, 1988), but it did not stimulate [^{3}H]cyclic AMP accumulation (Table 2) and it has previously been found to be inactive in inducing excessive grooming behavior (Gispen et al., 1975).

The finding that ACTH-(1–24) more potently influenced adenylate cyclase activity in septal slices than in striatal slices, but did not significantly affect adenylate cyclase activity in hippocampal and cortical slices, fits with data concerning the occurrence and quantity of ACTH/MSH binding sites in these brain regions (striatum, moderate to high; septum, high; hippocampus and cortex; no detectable binding sites) as reported by Tatro (1990).

We previously reported that ACTH-(1–24) enhances the release of dopamine from septal slices, an effect probably involving a presynaptic mechanism (Florijn and Versteeg, 1989). Possibly, the ACTH/MSH receptor–coupled adenylate cyclase is located on dopaminergic terminals. We tested this possibility by means of unilaterally lesioning of the nigrostriatal dopamine system. The disappearance of dopamine, indicating destruction of dopaminergic terminals, from the left striatum did not appear to affect the magnitude of [Nle^4,D-Phe^7]α-MSH–induced [^{3}H]-cyclic AMP production, suggesting a postsynaptic localization of the adenylate cyclase-coupled ACTH/MSH receptor. However, the possibility that the putative ACTH/MSH receptor is located on astroglia (Van Calker et al., 1983; Zohar and Salomon, 1992) cannot be ruled out.

Excessive grooming can be modulated by the local administration of dopaminergic drugs in the striatum (Spruijt et al., 1986), which suggests a role of the dopaminergic system and of dopamine receptors in the display of this behavior. We have reported that ACTH-(1–24) acts as an apparent competitive inhibitor of the binding of [^{3}H]spiperone and [^{3}H]NPA to the dopamine D_2 receptor and, but less potently, inhibits the binding of various ligands to dopamine D_1, serotonin, muscarinic acetylcholine, excitatory amino acid receptors, and adrenoceptors as well (Florijn et al., 1991). We investigated the functional importance of this mechanism of action of ACTH-(1–24) on the dopamine receptor–mediated [^{3}H]cyclic AMP production. From Table 4 it is clear that even at a very high concentration of ACTH-(1–24), no change in the dopamine D_1 receptor–mediated adenylate cyclase activation or D_2 receptor–mediated adenylate cyclase inhibition is evident. After the addition of the dopamine D_2 receptor antagonist sulpiride, an increased [^{3}H]-cyclic AMP production was found, due to disinhibition caused by the blockade of the dopamine D_2 receptor. This effect of sulpiride and the effect of the dopamine D_2 receptor agonist quinpirole on dopamine D_1 receptor–mediated stimulation of adenylate cyclase activity were not affected by ACTH-(1–24). It is not likely, therefore, that the increased [^{3}H]cyclic AMP formation caused by ACTH-(1–24) involves an interaction with dopamine receptors.

Previously, a receptor-independent mechanism has been suggested to underlie the ACTH-induced display of excessive grooming, the inhibition of protein kinase C (PKC) by ACTH (Schrama et al., 1987). In synaptosomal plasma membranes, a direct inhibitory effect of ACTH-(1–24) on PKC has been found to lead to a diminished phosphorylation of the nervous tissue–specific protein B-50 and to changes in polyphosphoinositide metabolism (Jolles et al., 1980). Structure–activity relationship studies performed with a limited number of ACTH-like peptides, and tested at 0.1 m*M* concentrations, revealed that the structural requirements for the induction of excessive grooming in vivo and for the inhibition of the nervous tissue–specific protein B-50 phosphorylation in vitro were almost identical (Zwiers et al., 1978). However, α-MSH and its analogue, [Nle^4,D-Phe^7]α-MSH, were not tested by Zwiers et al. (1978), but were found to be inactive in our studies (P. N. E. de Graan, personal communication).

Taken together, our data provide evidence for the existence of a putative adenylate cyclase–coupled ACTH/MSH receptor in the brain. This receptor may be the primary site at which ACTH/MSH peptides act, leading to a cascade of biochemical events and resulting in the display of different behaviors.

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