NOTE

Gamma-MSH and its Related Peptides do not Augment ACTH-Induced Steroidogenesis in Isolated Rat Adrenal Cells

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Abstract

In a sensitive ACTH bioassay system using isolated rat adrenal cells, we tested the effect of γ-MSH related peptides on ACTH-induced steroidogenesis. Peptides, including synthetic γ1-, γ2-, γ3- and Lys-γ3-MSH, exerted no effect in augmenting ACTH-induced steroidogenesis. None of the 16 kilodalton fragment of ACTH/β-lipotropin precursor and its cleaved fragment had such an activity. The results are in contrast with previous reports concerning ACTH-potentiating activity of γ-MSH related peptides and, therefore, indicate the necessity of further investigation of the principle involved in this unique biological activity.

Proopiomelanocortin, the ACTH/β-endorphin precursor (Eipper and Mains, 1980), contains within its amino-terminal region an MSH-like peptide, denoted γ-MSH by Nakanishi et al. (1979). Several lines of evidence suggest that some of the γ-MSH related peptides are active in augmenting ACTH-induced steroidogenesis in adrenocortical cells (Pedersen and Brownie, 1980a and 1980b, Al-Dujaili et al., 1981 and Farese et al., 1983). We have demonstrated, in a bioassay system for ACTH, the presence of similar activities in an extract of rat anterior pituitary glands (Iida et al., 1981). The assay utilizes isolated rat adrenal cells and has been found to be very sensitive and reproducible in detecting the ACTH-potentiating activities (Iida et al., 1982a and 1982b and Moriwaki et al. 1982). Using the same assay methods, we have demonstrated and partially characterized ACTH-potentiating factors in porcine thymus (Iida et al., 1984) and in porcine thyroid (Matsuyama et al., 1984). Thus, we tested the effect of γ-MSH and other related peptides on ACTH-induced steroidogenesis in isolated rat adrenal cells prepared by the methods that were employed in our previous studies.

Materials and Methods

γ1-, γ2-, γ3-MSH and Lys-γ3-MSH were synthesized (Ling et al., 1979) and donated by Dr. N. Ling, Salk Institute for Biological Studies (La Jolla, California, U.S.A.). The 16-kilodalton fragment of ACTH precursor peptide obtained from the transplantable mouse pituitary tumor...
(AtT-20), the 16K peptide, was a gift of Dr. B. A. Eipper, Department of Physiology, University of Colorado (Denver, Colorado, U.S.A.).

Isolated rat adrenal cells were prepared as previously described (Iida et al., 1984). Quartered adrenals from 2 to 3 rats (Sprague-Dowley) were incubated in 5ml of Krebs-Ringer bicarbonate buffer containing 200 mg/dl glucose, 1 mg/ml collagenase (Worthington) and 50 μg/ml deoxyribonuclease (Sigma, type I) at 37°C under an atmosphere of 95% O₂: 5% CO₂. After incubating for 12 minutes, the medium was pipetted in and out 20 times with a plastic pipet. Isolated cells were collected by filtering through nylon gauze (mesh #200). This procedure was repeated 3 times. After washing and incubation with 2 ml of the buffer containing 8 mg of lima bean trypsin inhibitor (Worthington) for 20 minutes, the cells were resuspended in Krebs-Ringer bicarbonate buffer containing 200 mg/dl glucose and 1.5% bovine serum albumin. Fifty μl of cell suspension (100,000 to 150,000 cells/ml, viability more than 90%) was transferred to a well on a Teflon microplate. Additives were added in 20 μl of the same buffer. All the peptides used were readily soluble in 0.1N acetic acid and the solutions were diluted at least 100 times with the incubation buffer just prior to the incubation. After incubation for 2 hours, 50 μl of incubation medium were transferred to a test tube containing 0.2 ml of ethanol and dried by heating in boiling water for 5 minutes. Corticosterone concentrations were measured by the competitive protein binding assay (Murphy, 1976).

Trypsinization of the 16K peptide was carried out by incubating 2 μg of the peptide in 0.1 ml of ammonium bicarbonate buffer (0.1 mM, pH 7.9) containing 2 μg of trypsin (Worthington) at room temperature over night. The reaction was terminated, after adding 0.1 ml of the same buffer containing 2 mg of lima bean trypsin inhibitor (Worthington), by heating in boiling water for 20 min. Each experiment was performed at least twice with similar results.

Results

γ₁-, γ₂- and γ₃-MSH at concentrations ranging from 0.01 to 1.0 nM exhibited no steroidogenic effect in isolated rat adrenal cells. In the presence of 14 pg/ml of ACTH-(1–24), none of the γ-MSHs augmented the steroidogenic effect of ACTH, as shown in Table 1 (A and B). In this assay system, partially purified porcine thymic extract augmented ACTH-induced steroidogenesis (Table 1, A). When the effect of Lys-γ₃-MSH, the 16 K peptide and its trypsinized fragment were tested, none of them exerted demonstrable augmentation of ACTH-induced steroidogenesis (Table 1, B, C and D). As shown in Figures 1 and 2, γ₁-, γ₂- and γ₃-MSH again had no effect on dose response curves of ACTH.

Discussion

Pedersen and Brownie (1980a) reported first evidence that the N-terminal portion of proopiomelanocortin and its cleaved fragment augmented the steroidogenic action of ACTH in isolated rat adrenal cells. They further demonstrated the ACTH-potentiating activity of γ₃-MSH in the same assay system (1980b). A similar augmenting effect of the circulating N-terminal portion of proopiomelanotropin, in a superfusion system, has also been reported by Al-Dujaili et al. (1981). In addition, Farese et al. (1983) reported the ACTH-potentiating activity of Lys-γ₃-MSH in rat fasciculata cells. The demonstration of Lys-γ₃-MSH binding sites in rat adrenal cortex by Pedersen and Brownie (1983) provided further evidence indicating biological activities of γ-MSH related peptides in the adrenal cortex.

However, upon scrutinizing these reports closely, it is evident that the peptide preparations employed in each study were different. This raises questions about the exact structure required for the activity. The study by Pham-Huu-Trung et al. (1982) indicated that γ-MSH had no ACTH-potentiating activity in guinea pig adrenal cells. Farese, who reported the potentiating activity of Lys-γ₃-MSH, found no activity in γ₃-MSH (personal communication). Thus, the notion
Table 1. Effect of γ-MSH related peptides on ACTH-induced steroidogenesis in isolated rat adrenal cells.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Corticosterone production (ng/10⁴ cells·2h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(n=4)</td>
</tr>
<tr>
<td>None</td>
<td>N.D.</td>
</tr>
<tr>
<td>Partially purified thymic extract#</td>
<td>N.D.</td>
</tr>
<tr>
<td>γ₁-MSH 0.01 nM</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>18.1 ± 1.8</td>
</tr>
<tr>
<td>0.1 nM</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>19.1 ± 1.6</td>
</tr>
<tr>
<td>1.0 nM</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>18.7 ± 1.4</td>
</tr>
<tr>
<td>γ₂-MSH 0.01 nM</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>22.0 ± 1.8</td>
</tr>
<tr>
<td>0.1 nM</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>21.0 ± 1.2</td>
</tr>
<tr>
<td>1.0 nM</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>20.4 ± 0.8</td>
</tr>
<tr>
<td>γ₃-MSH 0.01 nM</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>19.1 ± 1.4</td>
</tr>
<tr>
<td>0.1 nM</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>19.6 ± 2.6</td>
</tr>
<tr>
<td>1.0 nM</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>18.2 ± 1.4</td>
</tr>
<tr>
<td>(B) None</td>
<td>N.D.</td>
</tr>
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</table>

(B) (n=4) (n=4) (n=4) (n=4)

γ₃-MSH 0.01 nM

0.1 nM

1.0 nM

Lys-γ₃-MSH 0.01 nM

0.1 nM

1.0 nM

(C) None

N.D.

14.1 ± 1.1

35.8 ± 2.3

16K peptide 0.1 nM

N.D.

14.2 ± 4.0

32.2 ± 4.9

1.0 nM

N.D.

17.5 ± 1.5

33.7 ± 2.1

(D) None

N.D.

11.2 ± 1.8

25.7 ± 6.6

Trypsinized 0.1 nM

N.D.

9.0 ± 3.1

27.3 ± 3.5

16K peptide

1.0 nM

N.D.

12.5 ± 2.1

23.0 ± 2.8

Values represent Mean ± S.D., N.D.=not detectable, * P<0.01 vs. ACTH-(1-24) only.

** Concentrations before treatment with trypsin. # Fraction I obtained through gel-filtration of the crude extract of porcine thymus as described previously (Iida et al., 1984) was used. Experiments (A) to (D) were carried out on different occasions.
Fig. 1. The effect of \( \gamma_1 \)- and \( \gamma_2 \)-MSH on ACTH-induced steroidogenesis in isolated rat adrenal cells. Each point indicates the mean of quadruplicate determinations.

Fig. 2. The effect of 0.1 and 1.0 nM \( \gamma_2 \)-MSH on ACTH-induced steroidogenesis in isolated rat adrenal cells. Each point indicates the mean of quadruplicate determinations.
that the ACTH-potentiating activity resides in γ-MSH related peptides requires closer examination in connection with the nature of the active principle.

In the present study, neither γ-MSHs nor its related peptides, including γ3-MSH, Lys-γ3-MSH and the 16 K peptide, caused an augmentation of ACTH-induced steroidogenesis in isolated rat adrenal cells. The trypsinized fragment of the 16 K peptide, reported by Pedersen and Brownie (1980a) to possess potentiating activity, lacked this effect in the present study. The concentrations of these peptides used in the present study ranged from 0.01 to 1.0 nM. These concentrations were close to those used in earlier studies by different authors. By using this cell preparation, we were able to demonstrate the ACTH-potentiating activities in extracts of various tissues (Iida et al., 1981, 1982a and 1982b). Our previous studies indicate that the factors, detected in porcine thymic extracts, are peptide in nature and present in different molecular weight forms (Iida et al., 1984). Therefore, the results presented here suggest that the activity, detected by the assay system employed in the present study, resides in peptides distinct from γ-MSHs, although the possibility exists that the extracts contained γ-MSH-like peptides with minor modifications. The present study indicates the necessity of further investigations on peptides having this unique biological activity.

References


Pedersen, R. C. and A. C. Brownie (1980a). Adrenocortical response to corticotropin is poten-


Pedersen, R. C. and A. C. Brownie (1983). Lys-

\( \gamma \)-melanotropin binds with high affinity to the rat adrenal cortex. Endocrinology 112, 1279–1287.