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The Melanotropin Potentiating Factor and β-Endorphin do not Modulate the α-Melanotropin- or Adrenocorticotropin-Induced Corticosteroidogenesis in Purified Isolated Rat Adrenal Cells

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Abstract—The ability of two pro-opiomelanocortin-derived peptides, the melanotropin potentiating factor (MPF) and β-endorphin (βEP), to affect corticosteroid production was studied in purified isolated rat adrenal cells. Addition of MPF or βEP, in doses from 5 pg to 5 μg, alone did not result in a corticosterone production. Furthermore, no effect of MPF or βEP in doses from 5 pg to as high as 5 μg for both peptides upon the ACTH or α-MSH-induced corticosterodogenesis was observed ($p > 0.1$). It is concluded that both MPF and βEP do not influence the steriodogenic activity in the adrenal gland. Use of these peptides for discrimination of the ACTH/α-MSH receptor interactions is suggested.

Introduction

The pigmentary activity of α-Melanocyte stimulating Hormone (α-MSH) and related peptides can be potentiated by simultaneous administration of the COOH-terminal end of human β-Lipotropin (hβLPH) as measured in the Anolis skin bioassay. This so-called melanotropin potentiating factor (MPF) has been identified as the amino acid sequence Lys-Lys-Gly-Glu i.e. $h\beta LPH^{88-91}$ (1). Alpha-MSH shares its amino acid sequence with adrenocorticotropin (ACTH), as is $ACTH^{1-13}$. Therefore, ACTH has the message for pigment dispersion in common with α-MSH. Besides its melanotropic activity in lower vertebrates ACTH exercises mainly corticosteroidogenic activity in mammals. Due to the presence of the bioactive part of ACTH, α-MSH is able to stimulate corticosteroidogenesis as well (2-5). In this respect it is of interest to investigate the potentiating activity of MPF upon both the ACTH- and the α-MSH-induced corticosterone production in adrenocortical cells.

MPF is also a part of βEP (βLPH^{61-91}). This hormone has been reportedly shown to affect corticosteroidogenesis in crude rat adrenocortical cells stimulatorily (6, 7) as well as inhibitorily (7).

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Furthermore, very recently it was reported (8) that pharmacological doses of βEP could attenuate the in vivo cortisol response to exogenous ACTH. These data prompted us to study the effect of βEP and MPF upon the steroidogenesis with and without ACTH and α-MSH in our purified isolated rat adrenal system.

Materials and Methods

Peptides

Synthetic $hACTH^{1-39}$-revised sequence (9), 188 IU/mg as estimated previously (10)- and α-MSH were generous gifts from Dr W. Rittel and Dr P. Dessaulles (CIBA-Geigy Ltd, Basle), β-endorphin and LPH^{88-91}(MPF) were prepared by ICI (Alderley Park, Cheshire, UK) as described (1) and kindly donated by Dr J. S. Morley.

Preparation of preincubated, purified isolated rat adrenal cells

The preparation of preincubated, purified isolated cells has been described in detail previously (4). Briefly, the procedure is as follows. Adrenals of 5 male Wistar rats were freed of fat, cut into ten pieces and incubated at 37° C for 50 min under 95% O_2, 5% CO_2 in a solution of 10 ml Krebs-Ringer bicarbonate glucose (KRBG) containing 32 mg crude collagenase (Sigma type I) and 400 mg BSA (Sigma, A 6003). After disruption of the tissue by pipetting, the suspension (excluding the remaining large particles) was transferred to a cold 100 ml polyethylene tube. Two ml of buffer containing 0.5% BSA and 7.65 mM Ca (KRBGACa) were added to the large particles and the material was disrupted again. Both supernatants were combined and centrifuged at 100 g for 10 min (4°C). The supernatant was discarded and the pellet was washed twice in 10 ml KRBGACa. After the final centrifugation the cells were resuspended in 4 ml KRBGACa and purified by layering one ml upon 8 ml 5% BSA in KRBGCa. After 30 min the upper layer was removed by suction and the 5% BSA layer, which then contained the purified cells, was diluted appropriately. The purified cells (0.8 ml aliquots) were preincubated for 1 hour at 37°C under an atmosphere of 95% O_2, + 5% CO_2 after which ACTH diluent (0.1 ml of 0.9% NaCl, 0.5% BSA, pH adjusted to 3.5 with N HCl) was added together with MPF or β-endorphin doses (dissolved in 0.1 ml KRBGACa).

Subsequently the cells were incubated for two hours under the same conditions as during the preincubation. All observations were made in duplicate except stated otherwise.

Corticosterone was measured fluorometrically as described before (11).

Potency analysis

In each experiment complete log dose-response curves of ACTH and α-MSH were constructed. The potencies of the peptides were expressed as the relations of the reciprocals of their molar ED50 to the reciprocal of the molar ED50 of $hACTH^{1-39}$. The potency of 100 was assigned to human ACTH (12).

Statistical evaluation

In order to compare the results of the experiments performed on different occasions, the control values – i.e. the corticosterone quantity in tubes without MPF or β-endorphin – were assigned to a value of 1.00 in each separate experiment. Means are presented ± one standard deviation. Student's t-test was used to test the statistical significance of differences between groups.

Results

Figure 1 shows typical log dose-response curves for ACTH and α-MSH. It can be seen that the curve for α-MSH is characterized by a similar slope and similar maximal corticosterone production as the curve for ACTH. In 6 experiments a mean potency for α-MSH of 0.022 ± 0.004 was calculated, 5000 times lower than the potency of the ACTH moiety. Addition of EP or MPF (both in doses from 5 pg to 5 g) alone did not stimulate corticosterone production. No effect of MPF on the ACTH-induced corticosteroidogenesis could be observed either: MPF in doses varying from 5 pg to 5 μg did not affect the stimulating effect of ACTH (Fig 2a). The same result was obtained when its influence on the α-MSH-induced corticosterone production was studied (Fig 2b). Similar observations were made regarding the effect of βEP on the ACTH- and α-MSH-induced corticosteroidogenesis.

The table summarizes the results of 4 experi-

ments. In none of the tests was a steroidogenic effect evoked by MPF or βEP alone, nor was the steroidogenic activity induced by ACTH or α-MSH modified in any way ($p > 0.1$).

Discussion

The results demonstrate that neither the melanotropin potentiating factor nor β-endorphin are able to evoke corticosteroidogenesis by themselves (Fig 1). Moreover, neither peptide influences the corticosterone production elicited by ACTH or α-MSH in preincubated, purified isolated rat adrenal cells (Fig 2; Table).

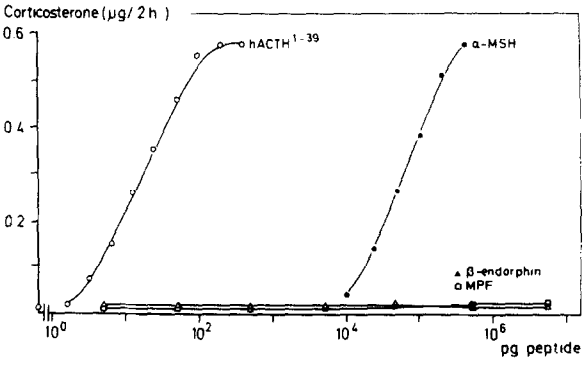

Fig 1 Log dose-response curves of the peptides studied.

It is well known that the corticosteroidogenic potency of α-MSH is much weaker than that of ACTH due to the lack of the basic amino acid sequence ACTH[15-18]. The potency of α-MSH in our system – calculated as about 5000 times lower than that of ACTH – is more or less in agreement with the values found in other studies (5, 13, 14). The presence of a second corticosteroidogenic "message" amino acid sequence within the ACTH molecule was recently demonstrated (15) and confirmed (16). Due to the low potency of fragments containing this second message it is believed that the main message sequence responsible for steroidogenesis is the 4-10 part of the molecule (12), of which Trp^9,His^6 and also Glu^5 are key elements for eliciting this bioactivity (17). Similarly, for the pigment dispersion in lower vertebrates two message sequences were found. Besides the central classical bioactive sequence 4-10, the tetrapeptide Gly-Lys-Pro-Val-NH_2 exhibits melanotropic activity (1) of α-MSH, but not the steroidogenic activity (Table) favors the idea that MPF potentiates only the second melanotropic active center, as it is ACTH/α-MSH[10-13]. The

Table Effect of MPF and β-Endorphin on the Relative Corticosterone Production[x], Basal or Induced by ACTH and α-MSH

Control	*Basal* 1.00	*ACTH (2.8×10^{-12}M)* 1.00	*α-MSH (1.5×10^{-8}M)* 1.00
α-endorphin			
1.5×10^{-11}M	1.11 ± 0.39	1.01 ± 0.09	1.07 ± 0.27
1.5×10^{-10}M	1.03 ± 0.12	1.91 ± 0.13	1.02 ± 0.14
1.5×10^{-9}M	1.12 ± 0.27	1.94 ± 0.09	0.98 ± 0.14
1.5×10^{-8}M	0.98 ± 0.05	1.02 ± 0.07	1.05 ± 0.18
1.5×10^{-7}M	1.04 ± 0.39	1.02 ± 0.08	1.06 ± 0.20
1.5×10^{-6}M	1.06 ± 0.07	1.00 ± 0.03	0.97 ± 0.22
MPF			
1.1×10^{-11}M	1.15 ± 0.36	1.03 ± 0.09	1.02 ± 0.26
1.1×10^{-10}M	0.98 ± 0.13	1.04 ± 0.12	0.98 ± 0.13
1.1×10^{-9}M	0.91 ± 0.19	0.96 ± 0.11	0.96 ± 0.15
1.1×10^{-8}M	1.04 ± 0.15	0.86 ± 0.09	1.02 ± 0.17
1.1×10^{-7}M	1.09 ± 0.16	1.04 ± 0.08	1.04 ± 0.17
1.1×10^{-6}M	1.17 ± 0.25	1.07 ± 0.06	1.03 ± 0.18
1.1×10^{-5}M	1.03 ± 0.18	0.97 ± 0.10	0.99 ± 0.17
1.1×10^{-4}M	0.91 ± 0.28	0.99 ± 0.09	1.03 ± 0.14

[x] The corticosterone productions in the different experiments were: 0.008; 0.002; 0.005 and 0.01 (basal), 0.079; 0.255; 0.125 and 0.168 (2.8×10^{-12}M ACTH), and 0.075; 0.148; 0.091 and 0.101 (1.5×10^{-8}M α-MSH) µg corticosterone respectively.

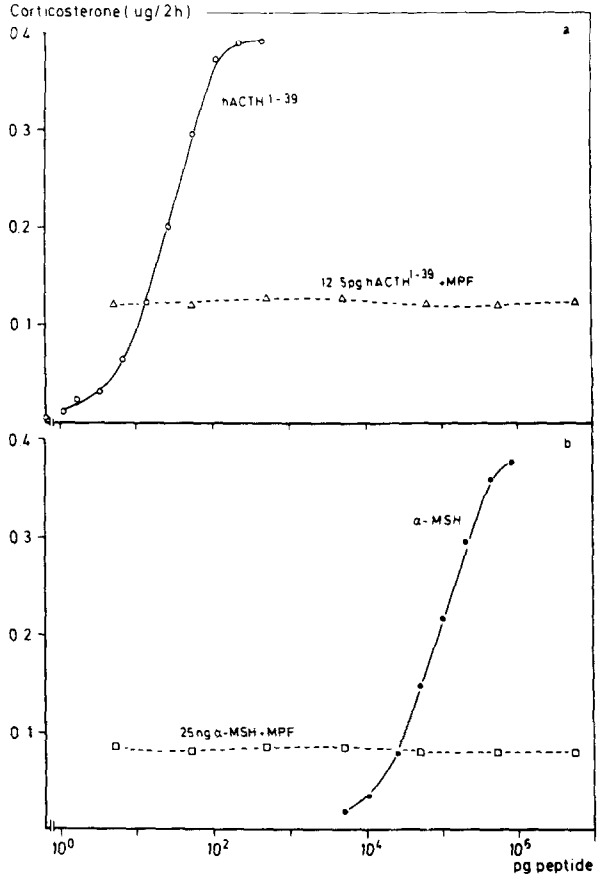

Fig 2a Log dose-response curve of hACTH and the effect of addition of MPF (doses 5 pg to 5 μg) upon the ACTH-induced corticosterone production.

b Log dose-response curve of α-MSH and the effect of MPF addition (doses 5 pg to 5 μg) upon the α-MSH-induced corticosterone production.

result of the present study is reminiscent of the findings of others (19). These authors demonstrated that the lipolytic activity of the melanotropic peptides α-MSH and β-MSH was not affected at all by MPF. It seems that MPF is able to potentiate MSH-like molecules when only the melanotropic activity is involved. With regard to this hypothesis it is worthwhile to study the effect of MPF on other activities of α-MSH, such as its influence upon behavioral adaptation (20, 21) or its ability to suppress basal and stress-induced prolactin release (22-24).

It is well known that βEP, βLPH and ACTH are derived from a common precursor: the pro-opiomelanocortin molecule (25, 26). Because concomitant secretion of ACTH and βLPH in the human (27, 28) and of ACTH and βEP in the rat had been demonstrated (29), the effect of βEP on the corticosteroidogenesis has been studied by several groups. Using isolated rat adrenal cells Szalay and Stark (7) found an inhibition of the basal corticosterone production in doses from 10^{-11} till 10^{-5}M, whereas 10^{-4}M stimulated the corticosterone production. Using the same technique Shanker and Sharma (6) also found that βEP could stimulate the corticosterone production, but only in concentrations of 5×10^{-9}M to 10^{-6}M, i.e. doses which evoked an inhibition in the study mentioned above (7). Moreover, these authors (6) found a maximal steroidogenic response of only one sixth of the maximal response induced by ACTH. Our study did not demonstrate an effect of this peptide at all. Similar findings had been reported previously (30) in bovine adrenal fasciculata cells. Likewise, discrepancies were found when the effect of βEP on the corticosterone production induced by ACTH was studied. Some authors (7) found an inhibition when concentrations between 10^{-9}M and 10^{-7}M βEP were tested. Using the same concentrations no inhibition has been observed in other studies (6, 30). In our study no inhibition by βEP could be detected (Table). It is important to stress that in contrast to the other studies we employed preincubated purified cells enabling estimation of potencies of peptides with more accuracy than in "crude" adrenal cell suspensions (11, 31).

This is the first study of the possible interaction between βEP and α-MSH upon the steroid synthesis in the adrenal cortex. The inability of βEP to modulate the α-MSH-induced corticosteroidogenesis (Table) supports the theory of similarity of action of both α-MSH and ACTH in adrenal activity (vide supra). It is of interest to note that very recently yet an antagonism between α-MSH and βEP was reported. Wardlaw et al. (32) showed that injection of relatively low doses of α-MSH together with βEP into the lateral cerebral ventricle decreased the prolactin release induced by injection of βEP alone. Bearing this in mind it would be worthwhile to do binding studies with receptors derived from different target organs of α-MSH/ACTH, in order to elucidate further the

interactions of the pro-opiomelanocortin-derived peptides.

References

1. Carter, R. J., Shuster, S. and Morley, J. S. (1979) Melanotropin potentiating factor is the C-terminal tetrapeptide of human β-lipotropin. Nature 279: 74-75.
2. Lowry, P. J., McMartin, C. and Peters, J. (1973) Properties of a simplified bioassay for adrenocorticotrophic activity using the steroidogenic response of isolated adrenal cells. J. Endocrinol 59: 43-55.
3. Sayers, G. (1977) Bioassay of ACTH using isolated adrenal cortex cells. Ann. N.Y. Acad. Sci. 297: 220-241.
4. Goverde, H. J. M. (1981) Bioactive versus immunoreactive adrenocorticotrophin in human blood. Ph.D. thesis, Catholic University, Nijmegen, pp 32-37.
5. Lambert, A., Frost, J., Garner, C. and Robertson, W. R. (1984) Cortisol production by dispersed guinea-pig adrenal cells; a specific, sensitive and reproducible response to ACTH and its fragments. J. Steroid Biochem. 21: 157-162.
6. Shanker, G. and Sharma, R. K. (1979) β-Endorphin stimulates corticosterone synthesis in isolated rat adrenal cells. Biochem. Biophys. Res. Commun. 86: 1-5.
7. Szalay, K. S. and Stark, E. (1981) Effect of beta-endorphin on the steroid production of isolated zona glomerulosa and zona fasciculata cells. Life Sci. 29: 1355-1361.
8. Beyer, H. S., Parker, L., Li, C. H., Stuart, D. and Sharp, B. M. (1986) β-Endorphin attenuates the serum response to exogenous adrenocorticotropin. J. Clin. Endocrinol. Metab. 62: 808-811.
9. Sieber, P., Rittel, W. and Riniker, B. (1972) Die Synthese von menschlichem adrenocorticotropem Hormon (α_h-ACTH) mit revidierter Aminosäuresequenz. Helv. Chim. Acta. 55: 1243-1266.
10. Schenkel-Hullinger, L., Main, R., Barthe, P. L., Dessaulles, P. A., Jarret, A., Riniker, B., Rittel, W. and Sieber, P. (1974) Biological activity of synthetic human corticotrophin with revised amino acid sequence. Acta Endocrinol. 75: 24-32.
11. Goverde, H. J. M., Pesman, G. J. and Benraad, T. J. (1980). Improved sensitivity to adrenocorticotrophin after purification and pre-incubation of isolated rat adrenal cells. Acta Endocrinol. 94: 221-227.
12. Schwyzer, R. Schiller, P., Seelig, S. and Sayers, G. (1971) Isolated adrenal cells: log dose response curves for steroidogenesis induced by $ACTH_{1-24}$, $ACTH_{1-10}$, $ACTH_{4-10}$, and $ACTH_{5-10}$. FEBS Letters. 19: 229-231.
13. Sayers, G., Swallow, R. L. and Giordano, N. D. (1971) An improved technique for the preparation of isolated rat adrenal cells: a sensitive, accurate and specific method for the assay of ACTH. Endocrinology 88: 1063-1068.
14. McMartin, C., Purdon, G. E. E., Schenkel, L., Desaulles, P. A., Maier, R., Brugger, M., Rittel, W. and Sieber, P. (1977) Differences between in-vitro and in-vivo potencies of corticotrophins: an interpretation in terms of metabolic stability. J. Endocrinol. 73: 79-89.
15. Goverde, H. J. M. and Smals, A. G. H. (1984) The anomalous effect of some ACTH-fragments missing the amino acid sequence 1-10 on the corticosteroidogenesis in purified isolated rat adrenal cells. FEBS Letters. 173: 23-26.
16. Szalay, K. S., de Wied, D., Stark, E. and Folly, G. (1985) Structure-activity studies with ACTH-α-MSH fragments on corticosteroid secretion of isolated zona glomerulosa and fasciculata cells. Reg. Pept. 11: 187-192.
17. Eberle, A. N., Kriwaczek, V. M. and Schulster, D. (1981) Corticotropin and melanotropin: Organization and transmission of hormonal information. In: Perspectives in Peptide Chemistry. (Eberle, A., Geiger, R. and Wieland T. eds). Karger, Basel, pp 407-422.
18. Eberle, A. and Schwyzer, R. (1975) Hormone-receptor interactions. Demonstration of two message sequences (active sites) in α-melanotropin. Helv. Chim. Acta. 58: 1528-1535.
19. Richter, W. D. and Schwandt, P. (1986) Melanotropin potentiating factor inhibits lipolytic activity of β-lipotropin but not of melanocyte stimulating hormones. Neuropeptides. 7: 73-79.
20. Bohus, B. (1984) Opiomelanocortins and behavioral adaptation. Pharmac. Therap. 26: 417-451.
21. O'Donohue, T. L. and Dorsa, D. M. (1982) The opionelanotropinergic neuronal and endocrine systems. Peptides. 3: 353-395.
22. Newman, C. B., Wardlaw, S. L. and Frantz, A. G. (1985) Suppression of basal and stress-induced prolactin release and stimulation of luteinizing hormone secretion by α-MSH. Life Sci. 36: 1661-1668.
23. Khorram, O., Bedran de Castro, J. C. and McCann, S. M. (1984) Physiological role of α-MSH in modulating the secretion of prolactin and luteinizing hormone in the female rat. Proc. Natl. Acad. Sci. USA. 81: 8004-8008.
24. Khorram, O., Deran de Castro, J. C. and McCann, S. M. (1985) Stress-induced secretion of α-melanocyte-stimulating hormone and its physiological role in modulating the secretion of prolactin and luteinizing hormone in the female rat. Endocrinology. 117: 2483-2489.
25. Mains, R. E., Eipper, B. A. and Ling, N. (1977) Common precursor to corticotropins and endorphins. Proc. Natl. Acad. Sci. USA 74: 3014-3018.
26. Nakanishi, S., Inoue, A., Kita, T., Nakamura, M., Chang, A. C. Y., Cohen, S. N. and Numa, S. (1979) Nucleotide sequence of cloned cDNA for bovine corticotropin-β-lipotropin precursor. Nature. 278: 423-427.
27. Gilkes, J. J. H., Bloomfield, G. A., Scott, A. P., Lowry, P. J., Ratcliffe, J. G. and Rees, L. H. (1975) Development and validation of a radioimmunoassay for peptides related to β-melanocyte-stimulating hormone in human plasma: the lipotropins. J. Clin. Endocrinol. Metab. 40: 450-457.
28. Krieger, D. T., Liotta, A. S. and Li, C. H. (1977) Human plasma immunoreactive β-lipotropin: correlation with basal stimulated plasma ACTH concentrations. Life. Sci. 21: 1771-1777.
29. Guillemin, R., Clayton, G. W., Vargo, T., Rossier, J., Minick, S., Ling, N., Rivier, C., Vale, W. and Bloom, F. (1977) β-Endorphin and adrenocorticotropin are secreted

concomitantly by the pituitary gland. Science. 197: 1367-1369.

30. Diel, F., Holz, J. and Bethge, N. (1981) Failure of somatostatin and β-endorphin to affect adrenal cortex cells in vitro. Horm. Metab. Res. 13: 95-98.
31. Bennett, H. P. J., Bullock, G., Lowry, P. J., Martin, C. and Peters, J. (1974) Fate of corticotrophins in an isolated adrenal cell bioassay and decrease of peptide breakdown by cell purification. Biochem. J. 138: 185-194.
32. Wardlaw, S. L., Smeal, M. M. and Markowitz, C. E. (1986) Antagonism of β-endorphin-induced prolactin release by α-melanocyte-stimulating hormone and corticotropin-like intermediate lobe peptide. Endocrinology. 119: 112-118.