Volume 296, number 1, 87–89 FEBS 10561 January 1992

Interaction of ACTH, β-endorphin and α-melanocyte stimulating hormone in relation to the corticosteroid production of isolated rat adrenocortical zona fasciculata and zona glomerulosa cells

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Received 1 November 1991

The combined effects of ACTH, β-endorphin (β-EP) and α-MSH were studied on the corticosteroidogenesis of isolated rat adrenocortical zona fasciculata and zona glomerulosa cells. β-EP potentiated the effects of ACTH and α-MSH on the zona fasciculata corticosterone production but inhibited those on the zona glomerulosa aldosterone production. β-EP did not affect the combined action of 4×10^{-11} M ACTH and 5×10^{-9} M α-MSH on the zona fasciculata or the zona glomerulosa cells, but it inhibited the stimulatory action of the combination of 1.6×10^{-10} M ACTH and 10^{-9} M α-MSH on the zona glomerulosa aldosterone production. An interaction of ACTH, β-EP and α-MSH in relation to the zona fasciculata and zona glomerulosa corticosteroid production was found.

ACTH; β-Endorphin; α-MSH; Proopiomelanocortin; Adrenocortical Steroidogenesis; Aldosterone

1. INTRODUCTION

Proopiomelanocortin (POMC)-derived peptides have different effects on adrenocortical steroidogenesis. β-EP is claimed to have stimulatory [1,2], inhibitory [3] or no effect at all [4] (for a review, see [5]). α-MSH in low doses stimulates the zona glomerulosa aldosterone production selectively [6,7] and potentiates the effect of ACTH on both the zona fasciculata corticosterone production and the zona glomerulosa aldosterone production [7]. As all of these peptides are present in the plasma, the question arises of how they act together on adrenocortical steroidogenesis.

ACTH, α-MSH and β-EP were added in different combinations and doses to isolated rat adrenocortical zona fasciculata and zona glomerulosa cells.

2. EXPERIMENTAL

Male CFY rats weighing 200–250 g were used. The rat adrenal cell preparation applied in our laboratory was described previously [8]. Briefly, cell suspensions were prepared by collagenase digestion of adrenal capsular strippings to yield zona glomerulosa cells, and of decapsulated adrenal glands to yield zona fasciculata cells. The zona fasciculata cell contamination in the zona glomerulosa cell suspension was less than 5%. In general, adrenal glands from 40 rats were digested for each adrenal cell preparation. The cells were prepared in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 2 g/l glucose and 40 g/l human serum albumin (HSA). Forty zona glomerulosa and 40 zona fasciculata (0.9 ml) cell suspension aliquots (approximately 3×10^5 cells/ml) were incubated in one session, in a shaking water-bath at 37°C under an atmosphere of 95% O_2 and 5% CO_2 for 2 h. ACTH (synthetic human αh^{1-39}-ACTH (National Institute of Arthritis and Metabolic Diseases, NIH, Bethesda, MD, USA), synthetic human β-EP (National Institute of Mental Health, Rockville, MD, USA) and α-MSH (Reanal, Hungary) were dissolved in physiological saline containing 5 g/l HSA and adjusted to pH 3.5. The concentrations of ACTH were 4×10^{-11} and 1.6×10^{-10} M, that of β-EP was 10^{-10} M and those of α-MSH were 10^{-9} M and 5×10^{-9} M. Experiments were performed in a randomized block format to eliminate bias due to systematic error. The experiments were carried out with 3 or 4 incubations in duplicate or triplicate at each dose.

The corticosterone contents of the incubation media (both zona glomerulosa and zona fasciculata) were determined by fluorimetry [9] after chloroform extraction. Aliquots of the chloroform extracts of the glomerulosa incubates were assayed for aldosterone content by radioimmunoassay without chromatographic separation [8].

Statistical analysis: 3-way analysis of variance was used for the logarithms of the data, and because of the significant interactions Dunn contrasts [10] were used to detect significant differences between means.

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3. RESULTS

Tables I and II show all the experimental data. β-EP (10^{-10}M) alone had no effect or slightly stimulated the zona fasciculata corticosterone production whereas it inhibited the zona glomerulosa aldosterone production. α-MSH alone and ACTH alone each stimulated the hormone production of both cell types. β-EP potentiated the effect of ACTH on the zona fasciculata corticosterone production but inhibited that on the zona glomerulosa aldosterone production. β-EP had no effect or slightly potentiated the effect of α-MSH on the zona fasciculata corticosterone production but inhibited that on the zona glomerulosa aldosterone production. β-EP did not affect the combined action of

Table I

Treatment	Zona fasciculata corticosterone (pmol/ml/2 h)	Zona glomerulosa aldosterone (fmol/ml/2 h)
vehicle	(A) 155.1 (12)* [5.044 ± 0.002]	(I) 6417 (13) [8.767 ± 0.03]
β-EP	(B) 160.2 (12) [5.076 ± 0.0009]	(J) 5938 (12) [8.689 ± 0.0025]
α-MSH$_1$	(C) 198.3 (12) [5.290 ± 0.0085]	(K) 14220 (12) [9.562 ± 0.0027]
β-EP + α-MSH$_1$	(D) 209.1 (14) [5.343 ± 0.0047]	(L) 12676 (13) [9.447 ± 0.0036]
ACTH$_2$	(E) 221.4 (12) [5.400 ± 0.019]	(M) 15530 (12) [9.651 ± 0.0093]
β-EP+ACTH$_2$	(F) 265.7 (12) [5.582 ± 0.029]	(N) 12535 (11) [9.436 ± 0.011]
α-MSH$_1$+ACTH$_2$	(G) 338.3 (12) [5.824 ± 0.011]	(O) 28234 (12) [10.25 ± 0.017]
β-EP+α-MSH$_1$+ACTH$_2$	(H) 335.1 (14) [5.814 ± 0.012]	(P) 27809 (13) [10.23 ± 0.0017]

Values geometric means of data; *n* is given in parentheses); [arithmetic means and SE of log data].
doses: β-EP = 10^{-10} M; α-MSH$_1$ = 5×10^{-9} M; ACTH$_2$ = 4×10^{-11} M.
$P<0.05$: G-D
$P<0.01$: A-C, B-D, E-F, E-G, F-H, A-E, B-F, C-G, D-H, I-J, K-L, I-K, J-L, M-N, M-O, N-P, I-M, J-N, K-O, L-P.

4×10^{-11} M ACTH and 5×10^{-9} M α-MSH on the zona fasciculata or the zona glomerulosa cells, but it inhibited the stimulatory action of the combination of 1.6×10^{-10} M ACTH and 10^{-9} M α-MSH on the zona glomerulosa aldosterone production.

4. DISCUSSION

Although the effects of β-EP and α-MSH on adrenocortical steroidogenesis have been studied in detail [3,6,7], there are scarcely any data on the combined effects of the POMC peptides ACTH, β-EP and α-MSH.

Similarly as demonstrated by our earlier results [7], α-MSH increased adrenocortical steroidogenesis in both the zona fasciculata and the zona glomerulosa cells. The sensitivity of the zona glomerulosa cells to α-MSH is higher: lower doses that do not affect the zona fasciculata corticosterone production increase the aldosterone production of the zona glomerulosa cells, and therefore a specific aldosteronotropic effect has been suggested [6,7].

The effect of β-EP on adrenocortical steroidogenesis is controversial; it depends on the functional state of the adrenocortical cells [5]. In our present experiments, the effect of 10^{-10} M β-EP on the zona fasciculata cells was stimulatory, while that on the zona glomerulosa cells was inhibitory, regardless of whether it was added alone or together with ACTH or α-MSH.

Goverde et al. [11] found no effect of β-EP on α-MSH or ACTH-induced corticosteroidogenesis in purified isolated adrenocortical cells. The discrepancy between their results and ours can be explained by the difference in the cell preparations used.

Numerous data have been reported on the interactions of POMC peptides in relation to other endocrine systems. Khorram and McCann [12] showed that α-MSH acts as an antagonist to β-EP in regard to the secretion of prolactin whereas it potentiates the effects of β-EP in stimulating growth hormone secretion and inhibiting LH secretion. However, the experiments of Wardlaw and Ferin [13] indicated that α-MSH antagonizes the action of β-EP on both pituitary prolactin and LH release in the primate. In the presence of α-MSH, 10^{-7} β-EP (which itself had no effect) stimulated somatostatin release [14]. α-MSH prevents the inhibitory effect of β-EP on sexual behaviour [15].

Our results show that the adrenocortical action of one POMC-derived peptide may be influenced by the simul-

Table II

Treatment	Zona fasciculata corticosterone (pmol/ml/2 h)	Zona glomerulosa aldosterone (fmol/ml/2 h)
vehicle	(A) 150.7 (13)* [5.016 ± 0.003]	(I) 2493 (12) [7.821 ± 0.0066]
β-EP	(B) 164.5 (12) [5.103 ± 0.0035]	(J) 2326 (12) [7.752 ± 0.0096]
α-MSH$_1$	(C) 182.3 (12) [5.206 ± 0.0062]	(K) 2678 (12) [7.893 ± 0.0097]
β-EP+α-MSH$_1$	(D) 185.4 (12) [5.223 ± 0.0030]	(L) 2092 (13) [7.646 ± 0.0041]
ACTH$_2$	(E) 536.9 (11) [6.286 ± 0.019]	(M) 8497 (12) [9.048 ± 0.0094]
β-EP+ACTH$_2$	(F) 665.6 (10) [6.501 ± 0.031]	(N) 6623 (12) [8.798 ± 0.014]
α-MSH$_1$+ACTH$_2$	(G) 714.0 (11) [6.571 ± 0.010]	(O) 9185 (12) [9.125 ± 0.035]
β-EP+α-MSH$_1$+ACTH$_2$	(H) 680.8 (12) [6.524 ± 0.014]	(P) 6920 (12) [8.842 ± 0.0028]

Values are geometric means of data; *n* is given in parentheses; [arithmetic means and SE of log data].
doses: β-EP: 10^{-10} M, α-MSH$_2$: 10^{-9} M, ACTH$_1$: 1.6×10^{-10} M
$P < 0.05$: G-H, I-J.
$P < 0.01$: A-B, A-C, B-D, E-F, E-G, A-E, B-F, C-G, D-H, K-L, I-K, J-L, M-N, O-P, M-O, I-M, J-N, K-O, L-P.

taneous secretion of the other peptides from the same precursor. This raises the possibility that differential posttranslational processing of POMC may serve as a regulator of adrenocortical steroid secretion. The potentiating effect of α-MSH, or both the potentiating and the inhibitory effect of β-EP on corticosteroidogenesis, may be of greater importance in various situations when there is a dissociation of ACTH, α-MSH and β-EP release. This can occur in certain forms of stress (e.g. emotional stress) when the neurointermediate lobe of the pituitary is stimulated [16], where POMC processing differ from that in the anterior lobe (the anterior pituitary corticotrophs process the POMC molecule to synthesize ACTH, β-LPH and to a lesser extent β-EP, while the intermediate lobe processes the molecule further to synthesize α-MSH and β-EP), or after dexamethasone pretreatment, when ACTH release is blocked, but α-MSH release is not affected [17].

In conclusion, we can only agree with Bertolini [18], who suggested 10 years ago that 'endorphins-enkephalins and ACTH-MSH peptides might have opposite and mutually balancing effects on several functions linked to the reactions of higher organisms to the changes of the environment...'.

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