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β-ENDORPHIN STIMULATES CORTICOSTERONE SYNTHESIS IN ISOLATED RAT ADRENAL CELLS

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SUMMARY

Studies are presented which demonstrate that β-endorphin induces corticosterone synthesis in isolated fasciculata cells. This activation of steroidogenesis has a lag period of 3 to 5 minutes and is cycloheximide-sensitive. The data suggest that β-endorphin exhibits steroidogenic activity by binding to the adrenocorticotropic hormone receptors of the cells.

INTRODUCTION

Studies by Mains et. al. (1) indicated that adrenocorticotropic hormone (ACTH) and β-endorphin are derived from a single common precursor polypeptide of 31,000 daltons present in the pituitary. Subsequent studies of Guillemin et. al. (2) demonstrated that ACTH and β-endorphin are secreted simultaneously by the pituitary gland. Based on these studies, it has been postulated (2) that the regulatory mechanisms involved in the secretion and biosynthesis of ACTH and β-endorphin are common and identical. This raises the possibility that β-endorphin, like ACTH, might influence adrenal steroidogenesis and thus pituitary-adrenal axis. In the present study, we have investigated the first possibility. We present here evidence that isolated rat fasciculata cells (3-5) secrete corticosterone synthesis in response to β-endorphin.

MATERIALS AND METHODS

The isolated adrenal cells were prepared by trypsin digestion (3-5). The method of incubation for ACTH, β-endorphin, or other appropriate agents was that

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already described (3,4). In general for each isolated adrenal cell preparation, adrenals from 16 rats were used and the cells from each adrenal gland (approximately 2 x 10^6 cells) were resuspended in 0.8 ml of Krebs-Ringer-bicarbonate buffer, pH 7.4, containing 4% albumin and 0.2% glucose. After incubating for 2 hours, the corticosterone was measured fluorometrically (6). Synthetic β-endorphin from Calbiochem (Molecular weight 3465.4) was used in these studies. ACTH, a United States Pharmacopeia Standard, was purchased from United States Pharmacopeia. All other chemicals were reagent grade and were obtained commercially.

RESULTS AND DISCUSSION

Fig. 1 shows that β-endorphin stimulates corticosterone production in a typical sigmoid concentration-response manner indicating the binding affinity of the enkephalin with the fasciculata cell receptors. The half-maximal steroidogenic concentration of β-endorphin ($40x10^{-9}$M) is 1000-fold higher than ACTH ($30x10^{-12}$M) indicating that β-endorphin is only 0.1% as effective as ACTH in activating corticosterone production. Furthermore, the maximal steroidogenesis obtained with β-endorphin is only one-sixth of ACTH. Nonetheless, the maximal steroidogenic concentration of β-endorphin causes a very significant (6-fold) increase of corticosterone formation. The isolated adrenal cell preparation (3-5) used in this study does not possess any ACTH-degrading activity (7). Therefore, in contrast to the adrenal slices or *in vivo* experiments, this cell preparation measures the potency of ACTH at the cell receptor level. Based on these criteria, the binding affinity of β-endorphin with adrenal cell receptors is 1000-fold lower than that of ACTH. An interesting observation of this study was that higher than maximal steroidogenic concentrations of β-endorphin caused a significant decline of peak corticosterone synthesis. There was almost a 50% reduction in corticosterone synthesis by 1 X 10^{-6}M of β-endorphin as compared to the value obtained with 2.2 x 10^{-7}M. This indicates an allosteric modification of the membrane receptor by β-endorphin. The level at which such a negative

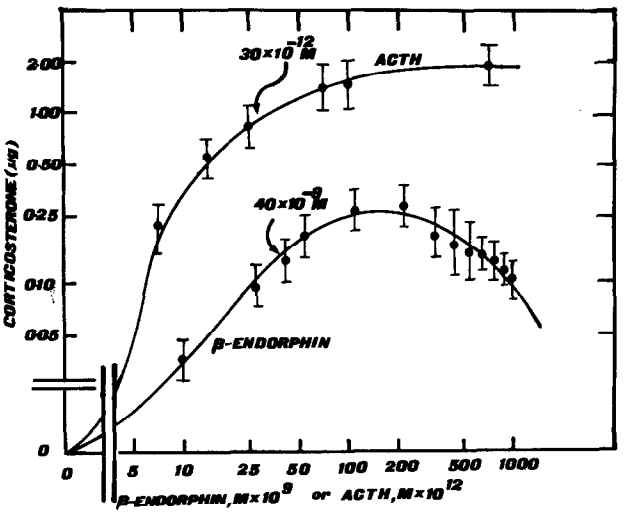

Fig. 1: Steroidogenesis in isolated fasciculata cells in response to β-endorphin and ACTH.

Incubation System: isolated adrenal cell suspension, 0.8 ml; reagents dissolved in 0.2 ml vehicle or Krebs-Ringer-bicarbonate buffer, pH 7.4, containing 4% albumin and 0.2% glucose. Total volume of incubation, 1 ml. Incubation was for 2 hours. Results are expressed as the mean values of 6 separate determinations from 3 different experiments. Control values have been subtracted from all experimental results. Concentration-response curves have been plotted on log-log scale.

cooperativity takes place cannot be identified at this time, since nothing is known about the nature of the interaction of β-endorphin with fasciculata cell receptors.

To answer the question of whether β-endorphin and ACTH activate steroidogenesis through the common or different receptors, corticosterone production was determined by incubating the cells with the combined maximal steroidogenic concentrations of these agents. The results obtained were not additive, suggesting that β-endorphin does not have its own distinct receptors but acts by combining with the ACTH receptors (Table 1). The alternative possibility is that

TABLE 1

Effect of Cycloheximide on the stimulation of steroidogenesis in isolated adrenal cells in response to β-endorphin and ACTH.

ADDITIONS	CORTICOSTERONE (μg/2 hrs)
Control	0.045
β-endorphin (2.2×10^{-7}M)	0.290
ACTH (100 μU/ml)	1.700
Cycloheximide (10 μM)	0.001
ACTH (100 μU/ml) + β-endorphin (2.2×10^{-7}M)	1.660
β-endorphin (2.2×10^{-7}M) + Cycloheximide (10 μM)	0.019
ACTH (100 μU/ml) + Cycloheximide (10 μM)	0.260
ACTH (100 μU/ml) + β-endorphin (2.2×10^{-7}M) + Cycloheximide (10 μM)	0.270

Incubation system: Isolated adrenal cell suspension, 0.8 ml; reagents dissolved in 0.2 ml vehicle or Krebs-Ringer-bicarbonate buffer, pH 7.4, containing 4% albumin and 0.2% glucose. Total volume of incubation, 1 ml. The mean values shown are derived from 6 observations from 3 different experiments. Control value has been subtracted from all experimental results.

β-endorphin and ACTH have separate receptors but common cyclase system. From these data, one could postulate that the steroidogenic-activating mechanism of β-endorphin is the same as that of ACTH. This indeed may be the case since analogous to the ACTH effect (3), β-endorphin-stimulated steroidogenesis has a definite lag period of 3 to 5 minutes (Fig. 2). This process also is cycloheximide-sensitive (Table 1). To date, the majority of evidence indicates that cyclic GMP and cyclic AMP components of the cell play an important mediatory role in ACTH-induced steroidogenesis (for a review see ref. 8). Based on the present data, one could postulate that these cyclic nucleotides also have a similar role in the propagation of β-endorphin action.

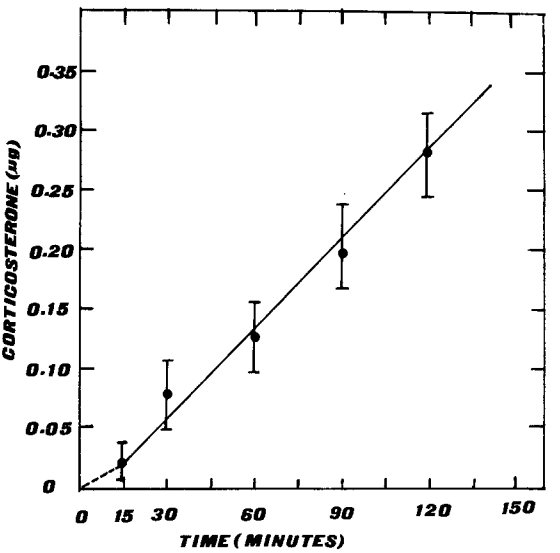

Fig. 2: Time course study of steroidogenesis in isolated fasciculata cells in response to β-endorphin. Conditions of the experiment were identical to Fig. 1.

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REFERENCES

1. Mains, R. E., Eipper, B. A. and Nicholas, L. (1977), Proc. Natl. Acad. Sci. U.S.A. 74, 3014-3018.

2. Guillemin, R., Vargo, T., Rossier, J., Minick, S., Ling, N., Rivier, C., Vale, W. and Bloom, F. (1977), Science 197, 1367-1368.

3. Kitabchi, A. E. and Sharma, R. K. (1971), Endocrinology 88, 1109-1116.

4. Sharma, R. K., Hashimoto, K. E. and Kitabchi, A. E. (1972), Endocrinology 91, 994-1003.

5. Sayers, G., Swallow, R. L. and Giordano, N. D. (1971), Endocrinology 88, 1063-1068.

6. Glick, D., Von Redlich, D. and Levine, S. (1964), Endocrinology 74, 653-655.

7. McMartin, C., Purdon, G. E. E., Schenkel, L., Desaules, P. A., Maier, R., Brugger, M., Rittel, W., and Sieber, P. (1977), J. Endocrinol. 73, 79-89.

8. Sharma, R. K. (1978), In: "Endocrine Control in Neoplasia" (eds. Sharma, R. K. and Criss, W. E.), pp. 13-52, Raven Press, New York.