Regulatory Peptides, 11 (1985) 187–192
Elsevier

RPT 00376

Structure-activity studies with ACTH/α-MSH fragments on corticosteroid secretion of isolated zona glomerulosa and fasciculata cells*

Katalin Sz. Szalay[a], D. De Wied[b], E. Stark[a] and G. Folly[a]

[a]*Institute of Experimental Medicine, Hungarian Academy of Sciences, P.O.B. 67, 1450 Budapest, Hungary, and* [b]*Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands*

(Received 11 December 1984; revised manuscript received 11 March 1985; accepted for publication 7 April 1985)

Summary

The steroidogenic action of ACTH/α-MSH fragments was studied on isolated zona glomerulosa and zona fasciculata cells dispersed by collagenase. ACTH-(4–7), ACTH-(6–10), ACTH-(4–10) and ACTH-(11–13) stimulated corticosterone production of the zona fasciculata and aldosterone production of the zona glomerulosa cells. ACTH-(7–10) was ineffective. ACTH-(4–7) appeared to be the most potent peptide of the tested fragments. None of the fragments affected the steroidogenic action of ACTH-(1–39).

It is suggested that similar to the melanotropic effect of α-MSH two 'message' sequences for adrenocortical stimulation exist in the α-MSH part of the ACTH molecule.

corticosteroid secretion; structure-activity studies; ACTH; α-MSH; zona glomerulosa; zona fasciculata

Introduction

α-Melanotropin (α-MSH) specifically stimulates zona glomerulosa [1,2] and potentiates the effect of ACTH on corticosteroid production both of zona fasciculata and

* Presented in part at the XIVth Acta Endocrinologia Congress, June 1983, Stockholm. Published in part as an abstract in Acta Endocrinol. (Kbh.) Suppl. 256, 309 (1983).

Address for correspondence: Dr. Katalin Sz. Szalay, Institute of Experimental Medicine, Hungarian Academy of Sciences, P.O.B. 67, 1450 Budapest, Hungary.

zona glomerulosa cells in doses which alone are ineffective [2]. The present experiments were designed to investigate which part of the molecule induces these effects. To this end the effect of synthetic ACTH/α-MSH fragments: ACTH-(4–7), ACTH-(6–10), ACTH-(7–10), ACTH-(4–10) and ACTH-(11–13) were studied on basal and ACTH stimulated aldosterone production of isolated zona glomerulosa cells and on the corticosterone production of isolated zona fasciculata cells.

Materials and Methods

Materials

Synthetic human $\alpha_h{}^{1-39}$-ACTH (ACTH-(1–39)) and aldosterone antiserum (Sheep 088) were supplied by the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, Maryland, U.S.A. The ACTH fragments were synthetized by the Scientific Development Group, Oss, The Netherlands. The following materials were purchased: α-MSH, Reanal, Hungary; collagenase (type I), Worthington Chemical Corporation, U.S.A.; TC Medium 199, DIFCO Labs., U.S.A.; [1,2-^{3}H]aldosterone, Amersham, Radiochemical Centre, England.

Animals

Male CFY rats, weighing 200–250 g, were used.

Preparation of cell suspensions

In our former publications we have described the rat adrenal cell preparation used in our laboratory [3,4]. Briefly, cell suspensions were prepared by collagenase digestion of adrenal capsular strippings to yield a preparation of zona glomerulosa cells and of decapsulated adrenal glands to yield zona fasciculata/reticularis cells [5]. Fasciculata cell contamination in the zona glomerulosa cell suspension was less than 5%. Generally in each incubation 80 adrenals were used for the preparation of zona glomerulosa, and 40 adrenals for the preparation of zona fasciculata cell suspensions. Cells were prepared in Krebs–Ringer bicarbonate buffer (KRBG) containing 2 g/l glucose and 40 g/l human serum albumin (HSA) with a potassium concentration of 3.6 mmol/l and finally resuspended in a mixture of Medium 199 and potassium free KRBG (2:1, v/v) containing HSA (5 g/l). Potassium concentration of the medium was 3.5 mmol/l. Aliquots of 0.9 ml of glomerulosa and fasciculata cell suspensions (approx. 3–3.5 $\times$ 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-(1–39) and ACTH fragments were dissolved in physiological saline containing 5 g/l HSA and adjusted to pH 3.5. The fragments were added to the cell suspension from 10^{-9} to 5×10^{-4} M concentrations (ACTH-(11–13) to 10^{-3} M). In the experiments where the possible potentiating effect of the fragments on ACTH-(1–39) was studied the following doses were applied: ACTH-(1–39), 2×10^{-11} to 1.3×10^{-9} M; ACTH fragments, 10^{-5} M. The fragments were tested in 2–3 incubations each dose in duplicate or triplicate.

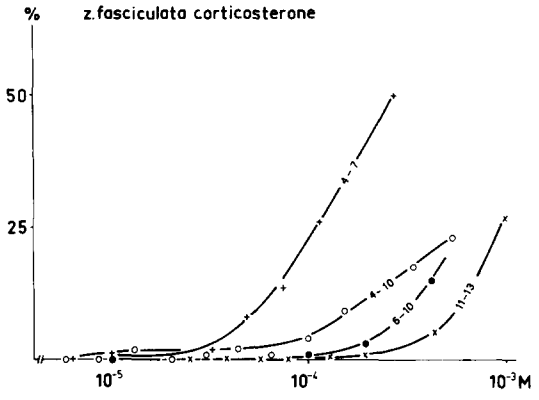

Fig. 1. Effect of ACTH fragments on corticosterone production of zona fasciculata cells. Mean ± S.E.M. basal production of corticosterone was 120 ± 10 pmol/ml. Results are expressed as percentage of the response obtained with 1.3×10^{-9} M ACTH-(1–39) (2354 ± 84 pmol/ml).

Steroid analysis

The corticosterone content of the incubation media (both from fasciculata and glomerulosa) was determined by fluorimetry [6] after chloroform extraction. Aliquots of the chloroform extract of the glomerulosa incubate were assayed for aldosterone content by radioimmunoassay without chromatographic separation [3].

Statistical evaluation

The dose-response curves were fitted by the logit-log method [7]. Potencies were estimated from fitted linear equations.

Results

Results are expressed as the percentage of the response obtained with 1.3×10^{-9} M ACTH-(1–39). (This dose was chosen, because it was the lowest which gave maximal effect.) All tested fragments with the exception of ACTH-(7–10) had a steroidogenic effect both on zona fasciculata and zona glomerulosa cells (Figs. 1 and 2).

ACTH-(4–7) appeared to be the most potent of the tested peptides both on zona glomerulosa and zona fasciculata cells. ACTH-(4–10) and ACTH-(11–13) affected the zona glomerulosa cells more potently than the zona fasciculata cells.

The ED_{50} was comparable for ACTH-(1–39) in the presence or absence of 5×10^{-4} M of the respective fragments. Thus, none of the fragments significantly affected the ACTH-(1–39) stimulated corticosteroidogenesis (Table I).

Discussion

The surprising finding in the present experiments is the steroidogenic effect of the fragments ACTH-(4–7), ACTH-(6–10) and ACTH-(11–13). To our knowledge, no

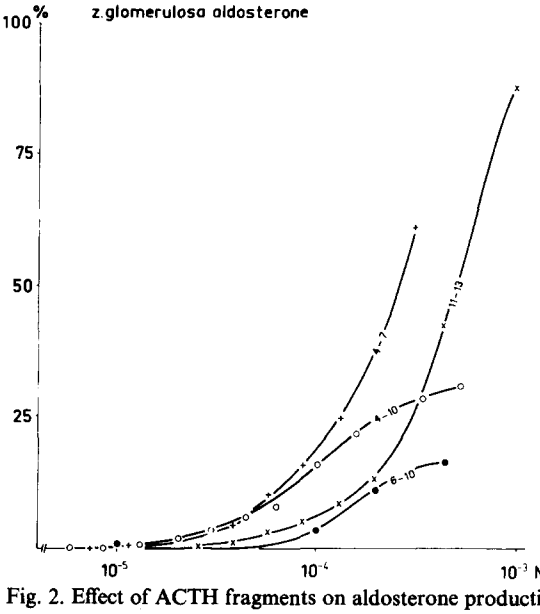

Fig. 2. Effect of ACTH fragments on aldosterone production of zona glomerulosa cells. Mean ± S.E.M. basal production of aldosterone was 5.1 ± 0.2 pmol/ml. Results are expressed as percentage of the response obtained with 1.3×10^{-9} M, ACTH-(1–39) (80 ± 6 pmol/ml).

TABLE I

ED_{50} of ACTH-(1–39) in the absence and presence of 10^{-5} M ACTH fragments

	ED_{50}	
	zona glomerulosa aldosterone	zona fasciculata corticosterone
ACTH-(1–39)	4.2×10^{-10}	2×10^{-10}
	(−21.6 ± 0.21)*	(−22.5 ± 0.06)
ACTH-(1–39) + ACTH-(4–7)	no data	2×10^{-10}
		(−22.3 ± 0.41)
ACTH-(1–39)	5×10^{-11}	4×10^{-11}
	(−23.7 ± 0.05)	(−23.9 ± 0.04)
ACTH-(1–39) + ACTH-(6–10)	5×10^{-11}	3.9×10^{-11}
	(−23.7 ± 0.07)	(−24.0 ± 0.01)
ACTH-(1–39)	6×10^{-10}	3.8×10^{-10}
	(−21.2 ± 0.13)	(−21.7 ± 0.13)
ACTH-(1–39) + ACTH-(4–10)	3.4×10^{-10}	4.2×10^{-10}
	(−21.8 ± 0.05)	(−21.6 ± 0.16)
ACTH-(1–39)	3.8×10^{-10}	3.3×10^{-10}
	(−21.7 ± 0.16)	(−21.8 ± 0.06)
ACTH-(1–39) + ACTH-(11–13)	3.6×10^{-10}	1×10^{-10}
	(−21.7 ± 0.06)	(−21.9 ± 0.12)

* ln ED_{50} and its S.D.

data have been published about the steroidogenic effect of these fragments. Schwyzer et al. [8] and Sayers et al. [9] reported that the shortest fragments with steroidogenic properties were ACTH-(4–10) and ACTH-(5–10). They used trypsin for isolating adrenal cortical cells. Although trypsinized cells can be effectively applied for the bioassay of the adrenocorticotrophic hormone [10], it has been recognized that trypsin can seriously affect the composition and properties of the cell surface and in this way the membrane receptors, while collagenase may be regarded as relatively innocuous to the cell membranes (see for review, Ref. 11). This may be pertinent to the difference between the present findings and those of Schwyzer et al. [8] and Sayers et al. [9].

The melanophore receptors may be triggered not only by ACTH-(4–10) but also by the C-terminal part ACTH-(11–13) [12]. Therefore 'message I' and 'message II' sequences were distinguished for the melanotropic effect of α-MSH [13]. However, according to Schwyzer et al. [8] the adrenal cortex could be triggered only by the main core, that is by ACTH-(4–10), or ACTH-(5–10), thus only one message sequence was supposed; however, they did not test shorter fragments. In the present experiments adrenal cortical cells could be stimulated also by ACTH-(11–13). Accordingly, we suggest that two message sequences exist also for steroidogenesis. The results of Löw et al. [14] can be taken as indirect evidence for supporting this assumption. These authors synthetized analogues of corticotropin-(1–19)-amide substituted with D-amino acids and measured their relative potencies in isolated adrenocortical cell suspensions. They found that the analogues D-Arg^{8} and D-Pro^{12} had markedly reduced activities.

The present data suggest that two parts of the α-MSH sequence are responsible for the glomerulotropic effect of this peptide. One important part seems to be the sequence ACTH-(4–7), which equipotently induced steroid production of zona fasciculata and zona glomerulosa cells. Thus, the receptors involved in this peptide-induced steroid production and present in the two zonae may be similar. It is striking that ACTH-(4–7) is more potent and displays a higher intrinsic activity than ACTH-(4–10) in which it is contained. At present we have no explanation for this phenomenon. Since ACTH-(6–10) had some activity, while ACTH-(7–10) was without effect, it may be suggested that the amino acid 6 is important for the effectiveness of ACTH-(4–7) in this respect. The other part is located within the 11–13 region of ACTH, but ACTH-(11–13) was less potent than ACTH-(4–7) in inducing steroid production. ACTH-(11–13) more potently affected the zona glomerulosa than the zona fasciculata cells, suggesting the involvement of different receptors. In former experiments [2] we demonstrated the ACTH potentiating effect of α-MSH both on zona glomerulosa and zona fasciculata cells. In the present experiments, however, the ACTH/α-MSH fragments did not show this ACTH-(1–39) potentiating effect. We suppose therefore that for this effect the whole sequence of the α-MSH molecule is required.

Acknowledgement

We thank Mrs. A. Falus for valuable technical assistance.

References

1 Vinson, G.P., Whitehouse, B., Dell, A., Etienne, T. and Morris, H.R., Characterisation of an adrenal zona glomerulosa-stimulating component of posterior pituitary extracts as α-MSH, Nature, 284 (1980) 464–467.

2 Szalay, K.Sz. and Stark, E., Effect of α-MSH on the corticosteroid production of isolated zona glomerulosa and zona fasciculata cells, Life Sci., 30 (1982) 2101–2108.

3 Szalay, K.Sz., Effect of pituitary intermediate lobe extract on steroid production by the isolated zona glomerulosa and fasciculata cells, Acta Physiol. Acad. Sci. Hung., 57 (1981) 225–231.

4 Szalay, K.Sz. and Stark, E., Effect of beta-endorphin on the steroid production of isolated zona glomerulosa and zona fasciculata cells, Life Sci., 29 (1981) 1355–1361.

5 Tait, J.F., Tait, S.A.S., Gould, R.P. and Mee, M.S.R., The properties of adrenal zona glomerulosa cells after purification by gravitational sedimentation, Proc. R. Soc. Series B, 185 (1974) 375–407.

6 Guillemin, R., Clayton, G.W., Lipscomb, H.S. and Smith, J.D., Fluorometric measurement of rat plasma and adrenal corticosterone concentration, J. Lab. Clin. Med., 53 (1959) 830–832.

7 Rodbard, D. and Frazier, G.R., Statistical analysis of radioligand assay data, Methods Enzymol., 37 (1975) 1–22.

8 Schwyzer, R., Schiller, P., Seelig, S. and Sayers, G., 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 Lett., 19 (1971) 229–231.

9 Sayers, G., Seelig, S. and Kumar, S., Isolated adrenal cortex cells in suspenson: stimulation and inhibition of steroidogenesis by analogues of ACTH, J. Steroid Biochem., 6 (1975) 371–375.

10 Sayers, G., Swallow, R.L. and Giordano, N.D., An improved technique for the preparation of isolated rat adrenal cells: a sensitive, accurate and specific method for the assay of ACTH, Endocrinology, 88 (1971) 1063–1068.

11 Waymouth, C., To disaggregate or not to disaggregate. Injury and cell disaggregation, transient or permanent?, In Vitro, 10 (1974) 97–111.

12 Eberle, A. and Schwyzer, R., Hormone-receptor interactions. Demonstration of two message sequences (active sites) in α-melanotropin, Helv. Chim. Acta, 58 (1975) 1528–1535.

13 Schwyzer, R., Organization and transduction of peptide information, Trends Pharmacol. Sci., (1980) 327–331.

14 Löw, M., Kisfaludy, L., Hajós, Gy., Szporny, L., Mihály, K., Makara, G.B., Toma, F., Dive, V. and Fermandijan, S., Biological and conformational properties of some corticotropin analogues containing D-amino acids. In K. Brunefeldt (Ed.), Peptides 1980, Scriptor, Copenhagen, 1981, pp. 513–519.