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RESPONSES OF RAT ADRENAL GLOMERULOSA AND INNER ZONE CELLS TO SYNTHETIC ACTH ANALOGS AND PROOPIOMELANOCORTIN-DERIVED PEPTIDES

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Summary—A comparison of the responsiveness of isolated rat adrenal decapsular and glomerulosa cells to corticotrophin 1–39 (ACTH 1–39), synthetic ACTH analogs (characterized by a shorter amino acid chain length, the substitution of certain amino acids in the natural sequence by other amino acid residues, the replacement of the C-terminal carboxyl group by an amide), and proopiomelanocortin-derived peptides was performed by measuring corticosterone and aldosterone production, respectively. The potencies of the synthetic ACTH analogs correlated closely with the length of the peptides, similarly in both zones. No activity was observed with the proopiomelanocortin-derived peptides in either zone, with the exception of β-LPH and α-MSH.

INTRODUCTION

Recent investigations have reported that the potency of porcine ACTH 1–39 in stimulating corticosterone and aldosterone production in rat decapsular and glomerulosa cells is comparable [1]. Studies with ACTH-related neuropeptides gave controversial results [2–14]. In the present study, we have used synthetic ACTH analogs and non-ACTH pituitary peptides derived from the same precursor, the proopiomelanocortin, to determine whether the glucocorticoid and mineralocorticoid stimulating activities of ACTH can be dissociated.

EXPERIMENTAL

Materials

Procine 1–39 ACTH was from Sigma. The various analogs, (D-Ser^1)-ACTH 1–24, (D-Ser^1, Lys$^{17-18}$) ACTH 1–19-OH, (D-Ser^1, Lys$^{17-18}$) ACTH 1–18-NH_2, ACTH 1–18-OH, ACTH 1–17-OH, ACTH 1–17-NH_2, ACTH 1–16-OH, ACTH 1–16-NH_2 were kindly provided by Dr Desaulles (Ciba-Geigy, Basel). The 16K fragment was donated by Drs Eipper and Mains, and β-LPH was a gift of Dr Li. Synthetic α-endorphin, β-endorphin, β-MSH, α-MSH, CLIP (Corticotrophin-like intermediate lobe peptide) were purchased from Bachem. FK 33–824, a sulfoxidecarbinol analog of methionine enkephalin, was a gift of Dr Del Pozo (Sandoz, Basel).

Methods

1. Preparation of isolated rat adrenal cells. Female Wistar rats (200–250 g) were maintained on normal laboratory diet containing 0.25% NaCl with tap water *ad libitum*. The animals were killed by decapitation and the adrenals were quickly removed. Glomerulosa and inner zone cells were prepared by collagenase digestion according to the method of Sala *et al.*[15]. The cells were resuspended in Medium 199 containing 0.2% of bovine serum albumin and 5.0 mM KCl.

2. Incubations. Cells (1 ml aliquots) were incubated in duplicate in polyethylene tubes for 2 h, in a shaking water bath at 37°C under an atmosphere of 95% O_2 and 5% CO_2, in the presence of various concentrations (10^{-11} M–10^{-6} M) of the ACTH analogs. Each peptide was tested in 4–8 separate experiments. The cell density was about 100,000 cells/ml for decapsular cells and 250,000 cells/ml for glomerulosa cells. At the end of the incubation period the cells were centrifuged and the supernatants were kept frozen until assayed for corticosterone and aldosterone.

3. Measurement of corticosterone and aldosterone. Corticosterone was measured by a direct radioimmunoassay, employing a rabbit antiserum raised in our laboratory against corticosterone-21-hemisuccinate–BSA conjugate, and Dextran charcoal separation. The validity of this assay was assessed by measurement of corticosterone after extraction with methanol. Regression analysis of the results obtained by direct assay and of those derived after methanol extraction, over a wide range of corticosterone values (1–500 ng) gave the following relationship: $y = 0.9877x + 1.2$ ($r = 0.996$, $P = 0.00001$, $n = 24$). Aldosterone was measured by direct radioimmunoassay of appropriate aliquots of the medium [16].

4. Data analysis. Radioimmunoassay data were analysed using a computer program based on the logit-log transformation of the standard curve [17].

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Table 1. The steroidogenic potency of ACTH analogs and proopiomelanocortin-related peptides

	Fasciculata-reticularis cells (M)	Glomerulosa cells (M)
ACTH 1–39	8.5×10^{-10}	2.5×10^{-9}
D-SER^1-ACTH 1–24	6.0×10^{-9}	2.5×10^{-9}
D-SER^1, LYS$^{17-18}$, ACTH 1–19 OH	10^{-9}	4.0×10^{-9}
D-SER^1, LYS$^{17-18}$, ACTH 1–19 NH_2	6.0×10^{-10}	2.5×10^{-9}
ACTH 1–18 OH	7.5×10^{-8}	7.0×10^{-9}
ACTH 1–17 OH	10^{-7}	7.0×10^{-8}
ACTH 1–17 NH_2	8.0×10^{-9}	10^{-8}
ACTH 1–16 OH	5.0×10^{-7}	10^{-7}
ACTH 1–16 NH_2	7.5×10^{-7}	9.0×10^{-7}
β-LPH	3.0×10^{-7}	1.5×10^{-6}

The values indicate the effective dose of each peptide which gave 50% of the maximal response measured with ACTH 1–39.

RESULTS

1. *Dose–response curves for ACTH* 1–39 *and various analogs*

In capsular cell suspensions, basal levels of aldosterone production were 98.6 ± 6.4 ng/10^5 cells (mean ± SEM of 8 experiments). Control values for corticosterone production by inner zone cells are 250.6 ± 30.0 ng/10^5 cells (mean ± SEM of 8 experiments).

The results of stimulation of glomerulosa and fasciculata-reticularis cells by increasing concentrations of the various peptides are shown in Fig. 1. To facilitate comparison between experiments, the results have been normalized and are expressed as percentage of the maximal steroid output induced by ACTH 1–39.

Since most of the peptides were not available in sufficient amounts to be used at concentrations above 10^{-7} M or 10^{-6} M, for most of them a plateau of maximal steroid production could not be reached; therefore, the ED_{50} could not be calculated. Table 1 indicates the doses of each peptide which gave a response corresponding to 50% of the maximal response induced by ACTH 1–39. ACTH 1–39 increased the production of aldosterone in glomerulosa cells 7-fold as compared with control levels, with an ED_{50} of 2.5×10^{-9} M. In fasciculata-reticularis cells, corticosterone production was stimulated 50-fold above controls, with an ED_{50} of 8.5×10^{-10} M. As shown in Fig. 1, ACTH 1–39 and its synthetic analogs produced very similar profiles in the dose-related stimulation of corticosterone and aldosterone production in rat decapsular and capsular cells. Porcine ACTH 1–39, ACTH 1–18, ACTH 1–17, ACTH 1–16 induced steroidogenic responses in isolated adrenal cells, with widely different potencies. The acidic forms were always less active than the corresponding peptides with a C-terminal amide. The replacement of the *N*-terminal L-serine by D-serine, and of the arginine at position 17–18 by lysine increased the apparent potency of the peptide.

Dose–response curves of proopiomelanocortin-derived peptides (Fig. 2)

No biological activity was observed with the 16K peptide, α-endorphin, β-endorphin, Met-enkephalin and CLIP in either zone. α-MSH at 10^{-6} M stimulated to the same extent both glomerulosa and inner zone cells, but β-MSH seemed more potent in glomerulosa cells. β-LPH displayed stimulatory effects at 10^{-6} M but contamination with ACTH could not be excluded.

DISCUSSION

In order to interpret these results, many factors which could explain the observed biological activity have to be considered: the relative affinities of the hormone analogs for the receptor, the rate of breakdown of the peptides, the time required for onset of the maximal response and possibly other variables. In attempts to assign definite potencies to a given analog or fragment, it is tacitly assumed that all peptides are affected in a quantitatively identical manner by these variables. In any case a comparison of the potency of these analogs on cells prepared in the same fashion from two zones of the same adrenal gland seemed justified. Our data are in agreement with previous results based on *in vivo* and *in vitro* assays [18–22]: a relationship between chain length and biological activity is again demonstrated. The major observation of our study is that the same pattern of response was found in both zones. Thus ACTH receptors whose activation is followed by aldosterone release from the zona glomerulosa cells appear to have the same stereospecificity as those of the zona fasciculata-reticularis involved in corticosterone production. The only ACTH analog reported to possess discordant aldosterone and corticosterone stimulating activities in rat capsular and decapsular cells was the synthetic (Cys(Cam)25)-α_h-ACTH(1–26) [23].

Recent *in vitro* studies with proopiomelanocortin-derived peptides have given controversial results. It has been reported that β-LPH and β-MSH selectively stimulated aldosterone production in collagenase dispersed capsular cells from rat adrenal glands [4,8,9]. In one study [12], a high concentration (10^{-7} M) of purified β-LPH from human and ovine pituitary gland was required to stimulate both aldosterone and corticosterone production *in vitro*. However, some of the steroidogenic activity of β-LPH was due in part

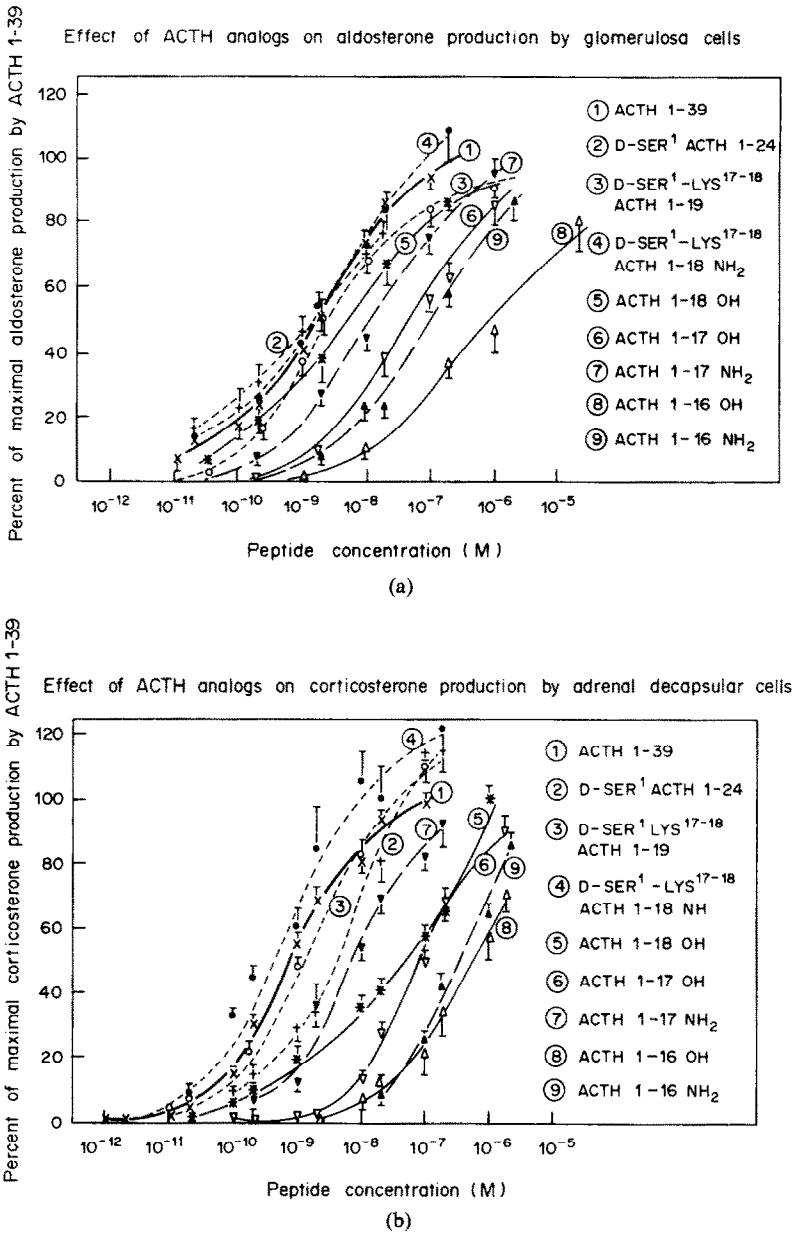

Fig. 1. Effect of ACTH analogs on aldosterone production by rat adrenal zona glomerulosa cells (upper) and corticosterone production by rat fasciculata-reticularis cells (lower). Each point is the mean ± SEM of duplicate determinations from 5–8 experiments.

to contaminating ACTH, and in part to structural similarities conferred by the heptapeptide core sequence common to ACTH, α-MSH, β-MSH, and β-LPH. Recently, Vinson *et al.*[14] showed that α-MSH stimulated B production in glomerulosa cells at concentrations at least 3 orders of magnitude lower than in fasciculata reticularis cells. Szalay and Stark[13] have found that α-MSH stimulated aldosterone, but not corticosterone in cells from normal rats. In contrast to Shanker and Sharma[2], and Szalay and Stark[11], but in agreement with Pedersen and Brownie[6], Matsuoka *et al.*[9], and Pham Huu Trung *et al.*[10], we did not observe any corticotropic activity for the synthetic α-endorphin. The inhibitory effect of enkephalins on steroid biosynthesis observed by Racz *et al.*[3] in the rat adrenal system was not confirmed by our data, which are consistent with those of Matsuoka *et al.*[9].

In conclusion, both glomerulosa and fasciculata-reticularis cells appear to possess ACTH receptors indistinguishable in terms of their stereo-specificity for a series of ACTH analogs and fragments. This observation, combined with our recent report of the similarity of the angiotensin receptor in both zones of the beef adrenal gland [24], suggests the notion that these cells might derive from a common precursor cell

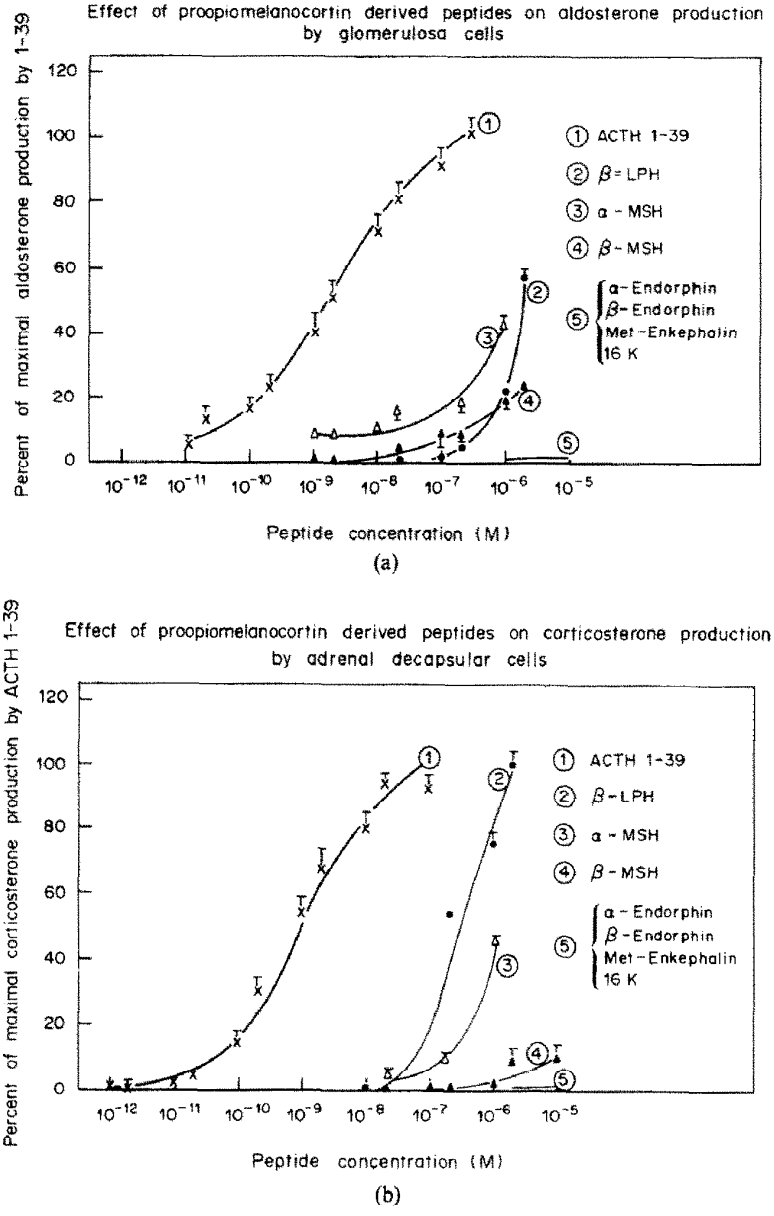

Fig. 2. Effect of proopiomelanocortin-derived peptides on the zona glomerulosa cell (upper) and the inner zone cell response (lower). Each point is the mean ± SEM of duplicate determinations from 5–8 experiments.

and maintain the same receptors on their membrane while differentiating in terms of the end product of the steroidogenic pathway in some species, while losing some receptors in other species.

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