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ADRENOCORTICOTROPIN 56. SYNTHESIS OF [CYS(CAM)^{25}]-α_h-ACTH-(1-26) AND BIS[CYS^{25}]-α_h-ACTH-(1-26). DISSOCIATION OF ALDOSTERONE- AND CORTICOSTERONE-STIMULATING ACTIVITY

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SUMMARY

Two analogs of human corticotropin (α_h-ACTH), [Cys(Cam)^{25}]-α_h-ACTH-(1-26) (I) and Bis[Cys^{25}]-α_h-ACTH-(1-26) (II), have been synthesized by modified procedures of the solid-phase method. When assayed for aldosterone production in rat capsular cells and corticosterone production in decapsular cells, it was found that peptide II had identical aldosterone- and corticosterone-stimulating activity as in the case of α_h-ACTH. However, peptide I was 6-7 times greater in stimulating aldosterone production in comparison with the stimulation of corticosterone production.

Recent investigations have shown that the concentration of ACTH (Fig. 1) required for half-maximal stimulation of aldosterone production is comparable to that concentration required for half-maximal stimulation of corticosterone production in rat adrenal cells (1). It is our intention to investigate synthetic ACTH analogs to determine if these two activities can be dissociated. We now report the solid-phase synthesis (2) of [Cys(Cam)^{25}]-ACTH-(1-26) (I) and show that the analog exhibits different aldosterone and corticosterone-stimulating activity in adrenal cells. In addition, the synthesis and properties of Bis[Cys^{25}]-α_h-ACTH-(1-26) (II), is also reported herein.

Abbreviations: ACTH, adrenocorticotropin, corticotropin; α_h-ACTH, human ACTH; CMC, carboxymethylcellulose; tlc, thin-layer chromatography; BSA, bovine serum albumin; Cam, carboxamidomethyl; Cm, carboxymethyl

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                          5                   10
NH2-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly

                     15                   20
Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-

                     25                   30
Lys-Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-

                     35              39
Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe-COOH
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Figure 1: Amino acid sequence of α_h-ACTH.

MATERIALS AND METHODS

The peptides [Cys(Cam)^{25}]-α_h-ACTH-(1-26)(I) and Bis[Cys^{25}]-α_h-ACTH-(1-26) (II) were each prepared from the same peptide resin. Boc-glycyl resin was alternately deblocked in 55% trifluoroacetic acid/methylene chloride, neutralized with diisopropylethylamine, and coupled with the symmetrical anhydride of the Boc amino acid as previously described (3). The protected peptide resin was treated with HF/anisole, and brief treatment by aqueous alkali at pH 11.5 removed the formyl group from the side chain of tryptophan to give the completely deprotected crude 26-peptide, which contained a cysteine residue at position 25. For preparation of peptide I, the crude peptide was treated with iodoacetamide, and the product was purified by chromatography on CMC (4) and partition chromatography on Sephadex G-50 (5). Peptide I was characterized by paper electrophoresis, tlc and amino acid analysis of acid and enzyme hydrolysates (Table 1). For preparation of peptide II, the monomer [Cys^{25}]-ACTH-(1-26) was purified as described for peptide I except that both CMC and partition chromatography were run in the presence of dithiothreitol to maintain the free -SH group of the cysteinyl side chain. The highly purified [Cys^{25}]-ACTH-(1-26) obtained from partition chromatography was stored in water at high concentration at pH 8.2. The course of the oxidation reaction was followed by analysis of aliquots with the Ellman reagent (6). After 2 days about 90% oxidation had occurred and the desired product (II) was separated from monomer by gel filtration on Sephadex G-50. Peptide II was characterized by paper electrophoresis and amino acid analysis of acid and enzyme hydrolysates (Table 1). The elution volume of peptide II compared to I on Sephadex G-50 was in agreement with peptide II having a dimeric structure.

Adrenal cells were prepared as described (7,8). Experiments were performed with cell incubations in triplicates in a final volume of 250 µl (11,000-22,500 cells) in polypropylene tubes at 37°C for 2 h under an atmosphere of 95% O_2 plus 5% CO_2 in a gyrotory water bath. Synthetic peptides were dissolved in 1 mM HCl and serially diluted with the incubation buffer in poly-

Table 1

Amino acid analysis of the synthetic peptides

Amino Acid	[Cys(Cam^{25}]-α_h-ACTH-(1-26)		Bis[Cys^{25}]-α_h-ACTH-(1-26)	
	Acid[a]	Enzyme[b]	Acid[a]	Enzyme[b]
Cys(Cm)	1.0 (1)[c]	-	-	-
Cys(Cam)	-	0.5[d] (1)	-	-
1/2 Cys	-	-	1.7 (2)	1.7 (2)
Ser	1.7 (2)	2.1 (2)	3.5 (4)	4.1 (4)
Glu	1.0 (1)	1.0 (1)	2.0 (2)	2.0 (2)
Pro	3.2 (3)	3.1 (3)	6.5 (6)	6.0 (6)
Gly	3.0 (3)	2.8 (3)	6.0 (6)	5.9 (6)
Val	2.9 (3)	2.9 (3)	5.9 (6)	6.0 (6)
Met	0.9 (1)	0.9 (1)	2.0 (2)	1.8 (2)
Trp	2.0 (2)	2.0 (2)	4.1 (4)	4.1 (4)
Phe	1.0 (1)	1.0 (1)	2.1 (2)	2.1 (2)
His	0.9 (1)	1.0 (1)	2.0 (2)	2.1 (2)
Lys	4.0 (4)	4.0 (4)	8.0 (8)	8.0 (8)
Trp	-	1.1 (1)	-	2.1 (2)
Arg	3.0 (3)	3.1 (3)	6.2 (6)	5.7 (6)

[a]Constant boiling HCl; 22 h at 110°C.

[b]Trypsin/chymotrypsin followed by leucineaminopeptidase.

[c]Numbers in parentheses are the theoretical values.

[d]Partially destroyed during enzymatic digestion; see (11).

propylene tubes. They were added in a volume of 25 µl and the rest of the volume was taken up by the addition of cell suspension. At the end of the incubation the tubes were frozen in a -20°C room.

Aldosterone was measured by radioimmunoassay using a rabbit anti-aldosterone-3-BSA serum (Miles Lab.). Corticosterone was also measured by radioimmunoassay using a specific antiserum characterized in this laboratory (9). For comparison of the steroidogenic activity of the synthetic analogs, synthetic α_h-ACTH (10) was employed as the standard.

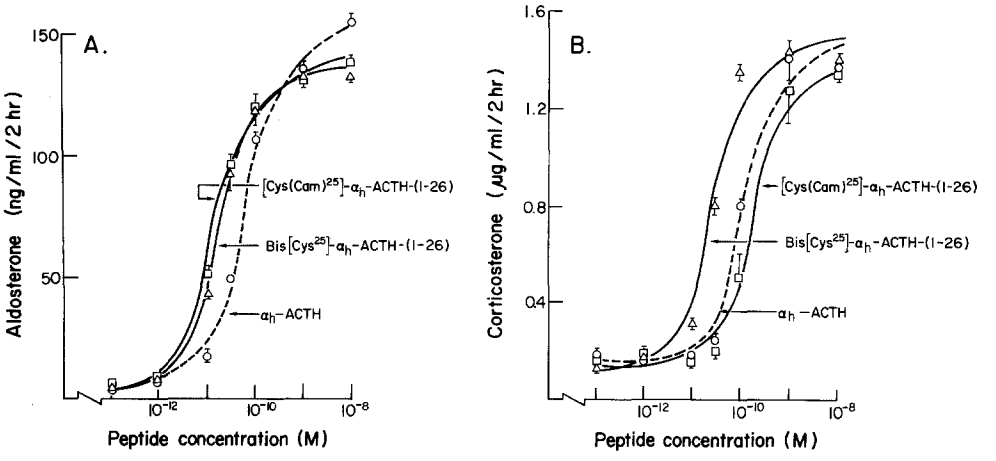

Figure 2: Effects of α_h-ACTH, [Cys(Cam)^{25}]-α_h-ACTH-(1-26) and Bis[Cys^{25}]-α_h-ACTH-(1-26) on the production of aldosterone (A) and corticosterone (B) in isolated rat adrenal capsular cells and cortical cells, Vertical bars extend to the limits of the SEM for triplicate incubations and analyses at each point.

RESULTS AND DISCUSSION

As shown in Fig. 2, α_h-ACTH and its synthetic analogs give very similar profiles in the stimulation of aldosterone and corticosterone production in the rat capsular and cortical cells, respectively. The dose-response curves are parallel and have the same maximal response. They differ only in the steroidogenic potencies. In the case of aldosterone production in capsular cells, peptides I and II have similar potencies ($P < 0.4$) and both of them are 3-4 times more active than α_h-ACTH. For corticosterone production in cortical cells, a different picture is observed. Peptide I is less active than α_h-ACTH but the differences are not significant ($P < 0.2$), and II is nearly 4 times more active than α_h-ACTH.

Since peptide II is a covalent dimer of I, 1 mole of II therefore contains 2 equivalents of I. Operationally, II is the same as I on a molar basis in the case of aldosterone

Table 2

Stimulation of steroidogenesis* of α_h-ACTH and synthetic analogs

	Aldosterone production in capsular cells			Corticosterone production in cortical cells		
	R_{max} (ng/ml/2h)	H_{50} (M)	RP (%)	R_{max} (μg/ml/2h)	H_{50} (M)	RP (%)
α_h-ACTH	152.0 ± 5.6	5.58 ± 0.91 x 10^{-11}	100	1.45 ± 0.11	9.30 ± 3.0 x 10^{-11}	100
[Cys(Cam)^{25}]-α_h-ACTH-(1-26)	137.4 ± 2.7	1.50 ± 0.15 x 10^{-11}	372	1.40 ± 0.10	16.0 ± 5.1 x 10^{-11}	58
Bis[Cys^{25}]-α_h-ACTH-(1-26)	135.5 ± 3.2	1.71 ± 0.20 x 10^{-11}	326	1.48 ± 0.09	2.43 ± 0.72 x 10^{-11}	383

*Values means ± SEM.

R_{max}, maximal response; H_{50}, half-maximal response; RP, relative potency

production in capsular cells. This could mean that only 1 out of the 2 equivalents of I in II is in operation at one time. In the case of corticosterone production in cortical cells, a different pattern is observed. II is several times more active than I. This could mean that both of the two I equivalents in II are in operation, and may even act cooperatively. Therefore, not only the structural requirements for the receptors could be different, the spatial arrangements of the receptors are possibly different in the two cell types as well. Nevertheless, it is evident that [Cys(Cam)^{25}]-α_h-ACTH-(1-26) exhibits only half of the potency of α_h-ACTH in stimulating corticosterone production in decapsular cells but is at least 3 times more active than α_h-ACTH in stimulating aldosterone production in capsular cells (see Table 2). As far as we are aware, this is the first report of a synthetic ACTH analog possessing diverse aldosterone- and corticosterone-stimulating activity.

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