Adrenocorticotropic Activity of Alpha Melanocyte Stimulating Hormone (α-MSH).* (25031)

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During the past 2 years α-MSH has been isolated and its structure determined(1,2). It is a tridecapeptide with an amino acid sequence identical to that of Corticotropin-A except that the N-terminal amino acid (serine) is acetylated and the C-terminal valine is in the amide form. A preliminary report of Lerner(3) stated that α-MSH did not possess corticotropic activity as measured by adrenal ascorbic acid method. Recently, Steelman *et al.*(4) have reported a simplified method for isolation of α- and β-MSH using carboxymethyl cellulose. Since ACTH-peptides have high adipokinetic activity(13, Steelman and Guillemin, to be published), α-MSH was assayed using the Payne(5) method. Doses as high as 1000 μg did not significantly increase liver fat in the mouse, whereas 1-3 μg of highly purified ACTH were active.

Materials and methods. ACTH activity of α-MSH(4) has been determined by the *in vitro* method of Saffran and Schally(6) and *in vivo* in 24 hr-hypophysectomized rat using levels of plasma free corticosteroids(7) and depletion of adrenal ascorbic acid(8) as criteria of adrenocorticotropic activity. In both types of assays USP Reference Standard Corticotropin was used as Standard. The *in vitro* assay is a 4-point design with duplicate observations for $U_{1,2}$ $S_{1,2}$; the *in vivo* assays were 4-point designs with 4 or 6 replicate observations per dose of each $U_{1,2}$ and $S_{1,2}$. The crude data of all assays were analyzed by variance analysis and factorial analysis with special emphasis placed on a study of degree of parallelism of the U/S log dose-response regression lines(9).

Results. Results are reported in Table I. All preparations of α-MSH tested exhibited corticotrophic activity. It appears that as degree of purification of α-MSH increases, so does corticotrophic activity. The corticotrophic activity was retained after incubation of the material at room temperature for 16 hr in 0.1N KOH or 0.1N HCl. Storage in deep freeze (−20°C) in dry state for 10 weeks did not alter significantly the corticotrophic or the melanophoretic activity.

Discussion. As reported elsewhere(4), the most purified MSH preparations used here exhibit less than 0.5 U/mg vasopressor activity when tested against the USP posterior lobe reference Standard. Assuming that this vasopressor activity is due to contamination with one of the vasopressins, evidence has been offered previously(7) which would preclude that the corticotrophic activity seen here could be attributed to either lysine or arginine vasopressin at the dose which could account for this contamination. The corticotrophic activity seen here seems to be truly inherent to the molecule of α-MSH.

Furthermore, in view of the absolute parallelism of the response curves, corticotrophic activity of α-MSH cannot be distinguished from that of ACTH USP Standard whether one measures Δ^4-3-keto steroids *in vitro,* plasma H_2SO_4-fluorescent steroids or adrenal ascorbic acid depletion *in vivo.* Considering such a parallelism of the otherwise linear log dose *vs.* response functions for unknowns and standard it appears that the corticotrophic activity of α-MSH cannot be related to the type of "non-specific" effects reported by Roberts(10) upon addition of large amounts of plasma proteins to adrenal fragments *in vitro;* the mathematical prerequisites for true corticotrophic activity as obtained here were never shown in the experiments reported by this author.

The discrepancy observed here between specific activities measured *in vitro* and *in vivo* is as yet not fully explained. A similar disparity has previously been reported for rat adenohypophysial extracts when tested

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TABLE I. Corticotrophic Activity of Several Preparations of α-MSH.

Material tested	Melanophoretic activity, U/mg*	Corticotrophic activity in USP units/mg with confidence limits of assays for p = .05		
		In vitro	*In vivo*	
			Plasma B	Adrenal ascorbic acid
α-MSH #6	3–5 × 10^6	1.8 (3.1– .4)		
α-MSH #6B (acid treated)	"	1.4 (2.4– .3)		
α-MSH #6A (alkali treated)	"	1.9 (6.1– .6)		
α-MSH #5D	"	1.7 (2.7–1.0) 1.2 (3.4– .5)		.11 (.30–.04)
" #14C	5 × 10^6	2.9 (10.8–1.4)	.12 (.13–.10)	.22 (.3 –.1)
" #21	1 × 10^7	3.5 (18.0–1.9)		
" #61B	"	3.6 (8.7–2.0)	.10 (.15–.04)	<.18

* *In vitro* assay of Shizume *et al.*(4). Melanophoretic activity expressed in units/mg.

against ACTH USP(11). The difference in activity was tentatively related to unequal rates of absorption by the adrenal tissue *in vitro* and unequal rates of inactivation and disappearance of the 2 substances *in vivo*(11, 12). Similarly the highly basic α-MSH could be tightly bound to blood or tissue proteins and rapidly become unavailable to the adrenal; or the acetyl-group on the N-terminus may reduce the affinity for the adrenal cortical tissue. It is also possible that (by enzymatically removing the acetylation of this N-terminus?) the adrenal tissue is capable of converting α-MSH to a more active corticotrophic molecule during the long contact *in vitro*.

In similar experiments, both *in vitro* and *in vivo*, no corticotrophic activity could be detected for β-MSH when it was tested in this laboratory against USP Reference Standard Corticotropin.

In view of the data presented here, it is suggested that the "active core" of the ACTH-molecule may be no larger than the tridecapeptide from the N-terminus.

Summary. Several preparations of highly purified α-MSH were studied for their adrenocorticotrophic activity *in vivo* and *in vitro* using USP Reference Corticotropin as a Standard. They showed corticotrophic activity by either assay procedure. β-MSH did not demonstrate similar activity. In view of data obtained, it is suggested that the "active core" of ACTH molecule may be no longer than the tridecapeptide from the N-terminus.

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