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On the properties of human β-melanocyte-stimulating hormone

β-Melanocyte-stimulating hormone from human pituitary glands has been found[1,2] to be a polypeptide containing 22 amino acid residues (Table I), in contrast to the MSH preparations isolated from pig[3,4] and ox[5] pituitaries, both of which contain 18 residues. Since even the porcine and bovine hormones, which are identical in length and almost identical in composition, differ in activity, the former being at least twice as potent[6] as the latter, and since no data has been reported concerning the melanotropic activity of β_h-MSH, it seemed of interest to ascertain the relative potency of the human hormone.

In the course of the isolation of human adrenocorticotropin, we have obtained a highly purified preparation of β_h-MSH. The starting material used in the present investigation was an oxycellulose concentrate[§] prepared from human pituitaries. Purified β_h-MSH was obtained by chromatography on CM-cellulose and DEAE-cellulose, with pH and concentration gradients essentially as described by SCHALLY *et al.*[7]; paper electrophoresis of the active material prepared in this way showed the presence of three components only one of which was active. For the final purification step, zone electrophoresis on paper was carried out in collidine acetate buffer of pH 6.9 for 18 h with a potential gradient of 12.5 V/cm at 5°. The peptide bands were eluted from the paper with 0.1 N acetic acid, and in this way 2.5 mg of highly purified β_h-MSH was obtained.

The melanocyte-stimulating potency of the preparation was determined by the frog-skin procedure *in vitro*[8] and found to be $3.3 \cdot 10^9$ U/g. When β_{Glu}-MSH (pig type) and β_{Ser}-MSH (beef type), isolated by similar procedures, were assayed, their potencies

TABLE I

STRUCTURE OF β-MSH ISOLATED FROM SEVERAL SPECIES

Species	*Amino acid sequence*
Human[1]	Ala–Glu–Lys–Lys–Asp–Glu–Gly–Pro–Tyr–Arg–Met–Glu–His–Phe–Arg–Try–Gly–Ser–Pro–Pro–Lys–A
Pig[3,4]	Asp–Glu–Gly–Pro–Tyr–Lys–Met–Glu–His–Phe–Arg–Try–Gly–Ser–Pro–Pro–Lys–A
Ox[5]	Asp–Ser–Gly–Pro–Tyr–Lys–Met–Glu–His–Phe–Arg–Try–Gly–Ser–Pro–Pro–Lys–A

TABLE II

AMINO ACID COMPOSITION OF CHYMOTRYPTIC PEPTIDES FROM β_h-MSH

*Peptide**	*Amino acid composition*	*Sequence expected from proposed structure***
1	Arg, 1.0; Try, 1.1	Arg–Try
2	His, 1.0; Arg, 1.1; Glu, 1.0; Met, 0.8; Phe, 0.9	Arg–Met–Glu–His–Phe
3	Lys, 1.0; Asp, 1.0; Ser, 1.0; Pro, 2.0; Gly, 1.0	Gly–Ser–Pro–Pro–Lys–Asp
4	Lys, 1.8; Asp, 1.0; Glu, 2.0; Pro, 1.1; Gly, 1.0; Ala, 1.1; Tyr, 0.9	Ala–Glu–Lys–Lys–Asp–Glu–Gly–Pro–T

* See Fig. 1.
** See ref. 1.

Abbreviation: β_h-MSH, β-melanocyte-stimulating hormone from human pituitary glands.

§ Part of the starting material was kindly supplied by Dr. M. S. RABEN.

were found to be respectively $3.8 \cdot 10^9$ U/g and $1.2 \cdot 10^9$ U/g. Thus, it would appear that the four additional amino acids of the human hormone have no effect in modifying the hormonal potency.

In order to ensure, as far as possible, that our preparation was identical with that used previously by other investigators[1,2], a number of chemical tests were made. A sample was hydrolyzed for 22 h with 5.7 N HCl at 110° in a sealed tube, and the hydrolyzate was examined on the automatic amino acid analyzer according to SPACKMAN, STEIN AND MOORE[9]. The molar ratio of the amino acids found was: Lys, 2.6; His, 1.1; Arg, 1.8; Asp, 1.9; Ser, 1.1; Glu, 2.9; Pro, 3.2; Gly, 2.3; Ala, 1.1; Met, 0.9; Tyr, 1.0; Phe, 1.0. In addition, minute amounts of valine and leucine were detected.

The remainder of the highly purified β_h-MSH (1.5 mg) was digested with chymotrypsin (0.05 mg) for 24 h at pH 8 and a temperature of 38°. The digest was subjected to paper electrophoresis: collidine acetate (pH 6.9), 12.5 V/cm, 5.5 h. When guide

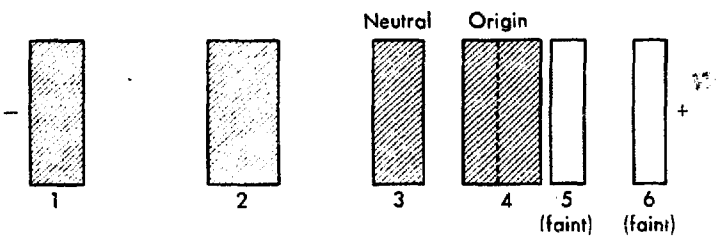

Fig. 1. Paper electrophoresis of a chymotryptic digest of β_h-MSH. Collidine acetate (pH 6.9), 12.5 V/cm, 5.5 h.

strips were stained with ninhydrin, four major and two minor bands were revealed (Fig. 1). The areas corresponding to these bands were eluted with 0.1 N acetic acid and lyophilized. The peptide obtained from band 1, suspected to be Arg–Try, was digested with leucine aminopeptidase, and those from the other bands were hydrolyzed with 5.7 N HCl. The amino acid compositions of the enzymic and acid hydrolyzates were then determined, with results as shown in Table II; peptides 1–4 had the amino acid compositions expected from the proposed structure[1].

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Hormone Research Laboratory, University of California, Berkeley, Calif. (U.S.A.)

B. T. PICKERING
CHOH HAO LI

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