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ISOLATION AND CHARACTERIZATION OF A NEW β-MELANOTROPIN FROM HORSE PITUITARY GLANDS

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SUMMARY

A new β-melanotropin was isolated from horse pituitaries and its primary structure has been determined. The amino acid sequence of the new peptide differed from that of β-melanotropin in the deletion of the NH_2-terminal aspartic acid. Thus, it is designated as [Des-Asp^1]-β-melanotropin. It possessed higher lipolytic activity when compared with β-melanotropin.

Isolation and amino acid sequence of α-MSH and β-MSH from horse pituitary glands have been reported (1,2). During the course of searching for β-lipotropin and β-endorphin in acid-acetone extract of horse pituitaries, we obtained a new peptide similar to β_e-MSH. Structural analysis of the peptide revealed it to be [Des-Asp^1]-β_e-MSH. When it was assayed in rabbit fat cells, it exhibited higher lipolytic potency when compared with β_e-MSH. Results of these studies are herein reported.

MATERIALS AND METHODS

Acid-acetone extracts of 300-g batches of whole horse pituitaries were obtained as described (3). The resulting acid-acetone powder from each batch was dissolved in distilled water and the solution was brought to 0.06 saturation with respect to NaCl and allowed to stand overnight at 4°. The precipitate that settled was removed by centrifugation and the supernatant was

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Abbreviations: MSH, Melanotropin; β_e-MSH, equine β-melanotropin; β_{c1}-MSH, camel β-melanotropin; HPLC, high performance liquid chromatography; CMC, carboxy-methylcellulose

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brought to saturation with NaCl. After standing overnight at 4°, the precipitate was dissolved in 0.1 N acetic acid and the solution was then desalted through a Sephadex G-10 column (3.5 x 80 cm, 0.1 N acetic acid). The desalted material was lyophilized. The lyophilized material (10 g from 12 batches; designated fraction D) was chromatographed on CMC as described (4). Fractions were pooled and lyophilized. Further purification of the fraction was achieved by chromatography on Sephadex G-25 fine, and HPLC. HPLC was carried out on a 10 x 500 mm column of Lichrosorb C8 (10 μ) with 10% n-propanol in pyridine acetate buffer of pH 5.5 at a flow rate of 1 ml/min as described (5).

Amino acid analysis was conducted according to the method of Spackman et al. (6) in an automatic amino acid analyzer (model 119C, Beckman Instruments). NH_2-terminal analysis was performed with the dansyl procedure (7,8) and the dansyl Edman method was employed for sequence analysis as previously described (7,9).

Electrophoresis on Whatman 3 MM paper was carried out in pH 6.7 collidine/acetic acid buffer (γ-collidine:HOAc:H_2O, 8.9:3.1:988, vol/vol) at 400 V. Trypsin (Worthington Biochemical) digestion was performed in 0.2 M NH_4OAc buffer (pH 8.5) at 37° for 4 hr with the enzyme/substrate ratio of 1:50. The tryptic peptides were subjected to paper chromatography on Whatman 3 MM paper with the upper phase of the system n-butanol:acetic acid: water (4:1:5, vol/vol) in the first dimension, followed by high voltage electrophoresis in the second dimension at pH 2.1 in 90% formic acid:acetic acid:water (218:63:79, vol/vol) for 45 min at 2 kV. The peptides were detected by spraying lightly with ninhydrin and then eluted from paper with 0.1 N NH_4OH. Appropriate aliquots were then submitted to amino acid and sequence analyses. Lipolytic activity was determined in vitro with rabbit fat cells as previously described (10).

RESULTS

Based on results from 12 separate experiments with 300-g horse pituitaries, the average yields from each extraction were 12 g of acid-acetone powder and 0.8 g fraction D. Figure 1 represents a typical chromatogram obtained when fraction D derived from 300-g horse pituitaries was chromatographed on CMC. The fractions were pooled and lyophilized as indicated. The yields of the fractions F and M were 24 mg and 25 mg, respectively.

Gel filtration of fraction M (200 mg) on Sephadex G-25 fine resolved it into 4 components; the most retarded component (12 mg) was further purified by HPLC as shown in Fig. 2 to yield

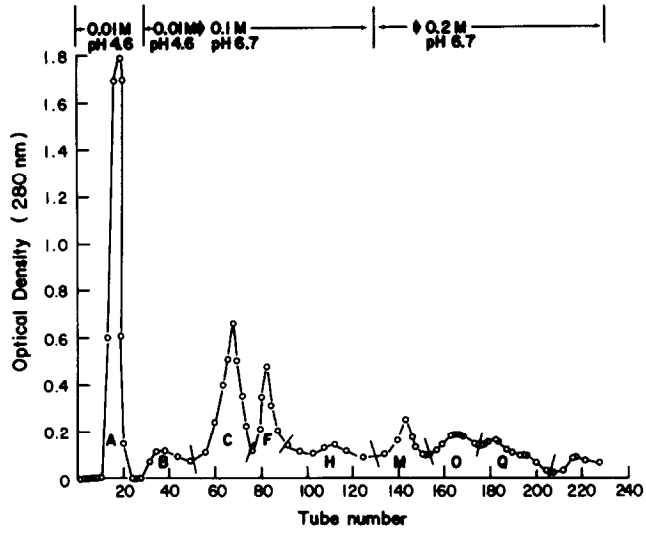

Fig. 1. CMC chrmoatography of 750 mg fraction D: Column size, 1.5 x 45 cm; flow rate, 12 ml/hr; 4 ml/tube. See text for conditions.

3.2 mg highly purified peptide M. When a sample of the purified peptide was run on paper at pH 6.7, it showed a single band with R_f = 0.31 relative to lysine. Its amino acid composition was

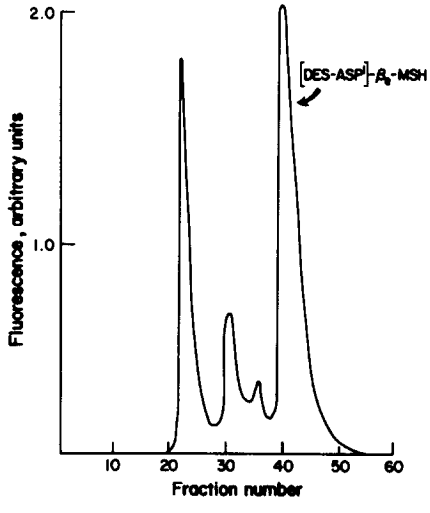

Fig. 2. Reverse-phase HPLC of 12 mg partially purified M obtained by gel filtration. The flow rate was 1 ml/min and 1 ml fractions were collected.

Table 1

Amino Acid Compositions[a] and NH_2-Terminal Residues of Purified Peptide M and Its Tryptic Peptides[b]

	T1	T2	T3	T4	Peptide M
Trp					(1)[d]
His			0.9		1.0 (1)
Lys	0.7	0.8			2.0 (2)
Arg			1.0	1.0	2.1 (2)
Asx	1.0				1.4 (1)
Thr					
Ser				0.8	0.9 (1)
Glx		0.7	1.2		2.1 (2)
Pro		0.9		1.0	2.0 (2)
Gly		1.0		1.0	2.2 (2)
Met			0.3[c]		1.0 (1)
Tyr		0.7			0.8 (1)
Phe			1.0		1.1 (1)
NH_2-terminal Residue	Lys	Glx	Met	Trp	Gly

[a]Molar ratios

[b]See Figure 3

[c]Low value due to oxidation of the Met during hydrolysis

[d]Detected by the Ehrlich test and assumed to be one residue

shown in Table 1. It had glutamic acid as its NH_2-terminal residue.

Figure 3 presents the tryptic peptide map of purified peptide M. The amino acid compositions and NH_2-terminal residues of the tryptic peptides are summarized in Table 1. The amino acid sequence of the peptide T2 was established to be: Glu-Gly-

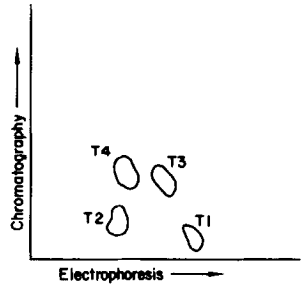

Fig. 3. Peptide map of tryptic digest of purified M (3 mg) obtained by HPLC.

Pro-Tyr-Lys. By homology with the sequence of β-MSH (2), the tryptic peptides of purified M are linked as: T2⟶T3⟶T4⟶T1. Thus, the amino acid sequence of purified M is: Glu-Gly-Pro-Tyr-Lys-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Arg-Lys-Asp-OH. It is evident that purified M is [Des-Asp^1]-β_e-MSH.

Using similar procedures, 36 mg β_e-MSH were obtained from 250 mg fraction F (see Fig. 1). The purified peptide showed a R_f of 0.2 relative to Lys at pH 6.7. The lipolytic activity of [Des-Asp^1]-β_e-MSH is presented in Table 2. For comparison, β_e-MSH was included in the assay using the synthetic β_{c1}-MSH (11) as the standard. Equine β-MSH was slightly more active while [Des-Asp^1]-β_e-MSH was about three times more active than β_{c1}-MSH.

DISCUSSION

The yield of [Des-Asp^1]-β_e-MSH was estimated to be 1.3 mg from 1 kg of fresh horse pituitary glands. This yield is much lower in comparison with that of β_e-MSH (11.5 mg/kg). The occurrence of [Des-Asp^1]-β_e-MSH in pituitary extracts has not been previously observed (12). As expected, it behaves as a more basic peptide than β-MSH in the elution profile on CMC chromatography as well as electrophoretic mobility at pH 6.7.

Table 2

Lipolytic Activity of [Des-Asp^1]-β_e-MSH

Hormone	Dose (ng)	Response[a]	Potency[b]		
			%	95% Confidence Limit	λ
β_{cl}-MSH	1.2	1.00 ± 0.54	100		
	3.7	2.33 ± 0.19			
	11.0	4.90 ± 0.56			
[Des-Asp^1]-β_e-MSH	0.4	0.40 ± 0.17	293	207-413	0.17
	1.2	2.35 ± 0.26			
	3.7	5.30 ± 0.43			
β_e-MSH	1.2	1.70 ± 0.23	150	93-263	0.25
	3.7	3.30 ± 0.27			
	11.0	5.38 ± 0.90			
	33.0	6.90 ± 0.31			

[a]Micromole of glycerol production per gram of cells per hour. Determinations in quadruplicate. Values in mean ± standard error.

[b]Statistical analysis of potency assuming β_{cl}-MSH as 100; λ, precision index.

Omission of NH_2-terminal Asp in β_e-MSH enhances its lipolytic activity (Table 2). In comparison with the primary structure of β_{cl}-MSH (13), there are differences in only 2 residues (camel: Gly-2, Pro-16; horse: Glu-2, Arg-16) in addition to the omission of Asp-1 in [Des-Asp^1]-β_e-MSH.

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