ISOLATION OF PEPTIDES WITH OPIATE ACTIVITY FROM SHEEP AND HUMAN PITUITARIES: RELATIONSHIP TO BETA-LIPOTROPIN

M. Chrétien, S. Benjannet, N. Dragon,
N.G. Seidah and M. Lis
Clinical Research Institute of Montreal.

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SUMMARY. Human and ovine pituitaries were found to contain peptides of identical size, composition and N-terminal residue to the carboxy-terminal portion 61-91 of beta-lipotropin (1-91). These peptides exhibit morphine-like activity in the mouse vas deferens bioassay. Beta-lipotropins from both species have much lower potency than the new peptides in the same bioassay. The findings favor the hypothesis that beta-lipotropin is the precursor of endogenous morphine-like peptides of pituitary origin.

Hughes et al. (1) recently isolated and described the structure of a pentapeptide from pig brain with potent morphine-like activity. This pentapeptide was given the name methionine-enkephalin (met-enkephalin) and it was also observed by Hughes et al. (1) to be structuraly identical to the 61-65 sequence of beta-lipotropin (beta-LPH) (2). In 1967, Chrétien and Li (3) discovered and described another peptide named gamma-lipotropin (gamma-LPH). It was found that gamma-LPH sequence is identical to the first 58 amino acids of beta-LPH. Moreover, both beta-LPH and gamma-LPH were found to contain in their 41-58 sequence the complete structure of beta-MSH. It was therefore proposed by Chrétie and Li (3), that beta-LPH is the prohormone of beta-MSH, gamma-LPH being an intermediate. The discovery of met-enkephalin raised the

possibility that beta-LPH could be its precursor as well. Li and Chung (4) isolated a peptide from camel pituitaries corresponding to 61-91 sequence of beta-LPH. Bradbury et al. (5) found an identical peptide in extracts from porcine pituitaries. All these peptides exhibit morphine-like activity. Finally, Seidah et al. (6, 7) have recently shown that the tryptic fragments 61-80, 61-81 and 61-91 of sheep beta-LPH have considerable morphine-like activity while beta-LPH (1-91) is much less active.

The present report describes the isolation and iden-- tification of peptides with morphine-like activity from both human and sheep pituitaries with amino acid composition corresponding to residues 61-91 of beta-LPH.

MATERIAL AND METHODS

Frozen human and sheep pituitaries were extracted according to the method of Li (8) and the extract chromatographed on either CM-cellulose or CM-sephadex.

Some of the human CM-cellulose fractions were further purified by Sephadex G-75 and eluted with 0.01 M ammonium acetate pH 4.6. The sheep pituitary extract was chromatographed on CM-Sephadex as described by Bradbury et al. (5) prior to the CM-cellulose step.

The morphine-like activity of isolated fractions was assayed on mouse vas deferens as described by Henderson et al. (9). The activity is expressed as percentage of inhibition of electrically stimulated contractions of mouse vas deferens in vitro. The maximal response is usually in the range of 70-80% inhibition.

Amino acid analysis was carried out according to Spackman et al. (10) using a Beckman 121C automatic analyzer. The N-terminal residue was identified by the dansyl procedure (11). Polyacrylamide gel electrophoresis was done according to Davis (12) at pH 4.5.

RESULTS

The CM-cellulose chromatography of human pituitary extract according to Li (8), is routinely used for purification of growth hormone, ACTH and beta-LPH (13). A slightly slower gradient revealed a new peak adjacent to that of growth hormone; the constituent possessed morphine-like activity. Gel filtration of this fraction

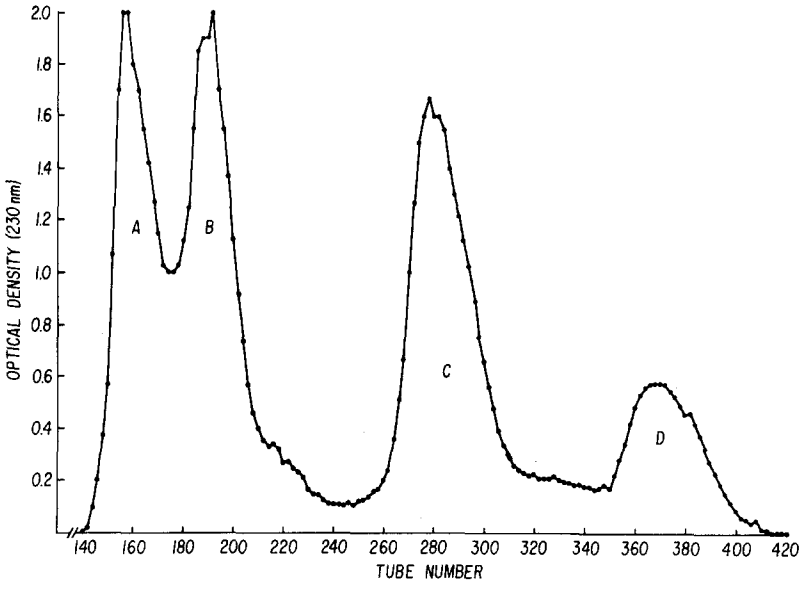

Figure 1: Purification of human fraction eluted from CM-cellulose (37.5 mg) possessing morphine-like activity on a column (2.5 x 120 cm) of sephadex G-75 superfine, using 0.01 M NH_4Ac pH 4.6 as eluant. Peak D gave the peptide 61-91 of human beta-LPH (see table I).

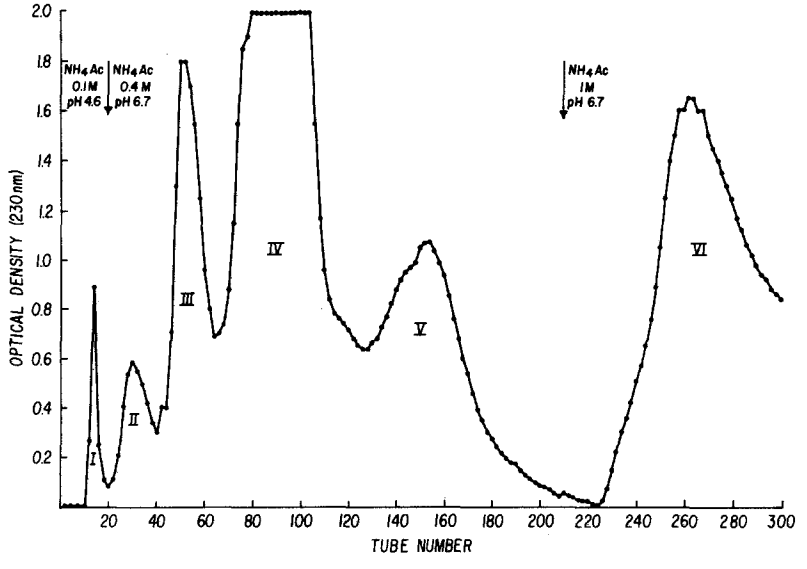

Figure 2: Purification of sheep pituitary extract eluted from CM-sephadex with 1 M NH_4Ac on a column (1 x 25 cm) of CM-cellulose, using a gradient of NH_4Ac from 0.1 to 1 M. Peak III, upon further purification on sephadex G-25, gave the peptide 61-91 of sheep beta-LPH (see table I).

TABLE I

AMINO ACID ANALYSIS OF HUMAN AND SHEEP 61-91 PEPTIDES

	Hydrolysis Time							
	Human*				Sheep*			
Amino Acid	24 h	48 h	72 h	Nearest Integer	24 h	48 h	72 h	Nearest Integer
Lys	4.53	5.28	4.80	5	4.96	5.34	5.00	5
His					0.91	0.91	0.90	1
Asp	2.08	2.22	2.06	2	2.28	1.93	2.09	2
Thr	2.71	2.61	2.28	3	2.98	2.49	2.59	3
Ser	1.84	1.58	1.32	2	1.94	1.53	1.51	2
Glu	2.99	2.76	3.21	3	3.01	2.67	2.78	3
Pro	1.20	1.09	1.21	1	1.32	1.23	1.11	1
Gly	3.27	3.24	3.01	3	3.00	2.66	2.75	3
Ala	2.20	2.06	1.98	2	2.32	2.08	2.06	2
Val	1.08	0.86	1.06	1	1.18	1.00	1.04	1
Met	0.67	0.40	0.79	1	0.91	0.83	0.73	1
Ile	1.28	1.34	1.73	2	1.32	1.69	1.82	2
Leu	2.03	1.80	2.11	2	2.25	2.13	2.15	2
Tyr	1.86	1.94	1.65	2	0.94	0.87	0.94	1
Phe	1.87	1.84	1.85	2	2.16	1.93	1.96	2

* Dansylation revealed that the N-terminal residue of both peptides is exclusively Tyr.
Preliminary results (Seidah, N.G. et al., unpublished results) showed that the complete sequence of both peptides is identical to the portion 61-91 of beta-LPH structure (8, 14).

on Sephadex G-75 is shown in Fig. 1. Peak D was found to contain most of the morphine-like activity. Its amino-acid composition corresponds to that of residues 61-91 of human beta-LPH (14) and the N-terminal residue is exclusively tyrosine (Table I).

The sheep pituitary extract eluted from CM-Sephadex 25 by 1 M NH_4Ac, was further purified on CM-cellulose as shown in Fig. 2. Repurification of the biologically active fraction yielded a pure material with amino-acid composition corresponding to the 61-91 fragment of sheep beta-LPH and with tyrosine as N-terminal residue (Table I). The polyacrylamide gel electrophoresis at

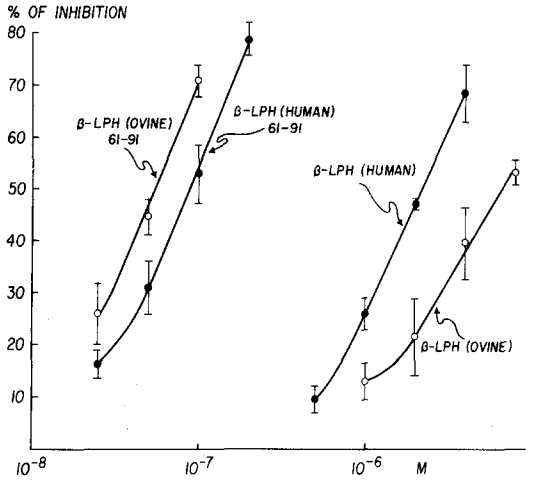

Figure 3: Morphine-like activity assayed on electrically stimulated mouse vas deferens. The activity is expressed as a % of inhibition of vas deferens contractions. All the points are the means of 4 measurements ± SE. Beta-LPH (ovine) 61-91 and beta-LPH (human) 61-91 mean that such peptides have the same amino-acid composition and the same N-terminal residue as the identified portion of both LPH's.

pH 4.5 showed an Rf 0.64 for human peptide and Rf 0.75 for sheep peptide.

The biological activity is shown in Fig. 3 where the morphine-like activities of both new peptides are compared to that of their respective beta-LPH (1-91) peptides.

DISCUSSION

Beta-LPH now appears to be a unique molecule comprising the structures of two biologically active peptides: beta-MSH and met-enkephalin. Although, there is no direct evidence showing that beta-LPH is the prohormone or precursor of both compounds, there are many indications which favor the prohormone theory proposed by Chrétien and Li in 1967 (3). The experimental findings supporting this theory could be summarized as follows:

Beta-LPH is a relatively stable molecule and the isolated beta-LPH fragments are not likely to be degradation products due to the isolation procedure, as shown by Chrétien and Gilardeau (15).

Chrétien et al. (16), using in vitro pulse-labelling techniques have already demonstrated the transformation of beta-LPH into gamma-LPH. Beta-LPH, gamma-LPH and the peptide (61-91) of beta-LPH are consistently isolated from pituitaries of different species (2-5) (17-19). Also, Guillemin et al. (20) recently isolated a fragment 61-76 from porcine neurohypophysis and hypothalamic tissue. Beta-LPH has much lower MSH activity than beta-MSH (8) and much lower morphine-like activity than the new peptides, which would be an analogous situation to the proinsulin - insulin model (21).

The different Rf values found for the human and sheep peptides on polyacrylamide gel electrophoresis at pH 4.5 could be explained by the fact that tyrosine replaces histidine in the human peptide.

The biological activity of both peptides is comparable. This is not surprising since they both contain met-enkephalin pentapeptide which, we believe to be the active core (6-7).

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