Proc. Natl. Acad. Sci. USA
Vol. 73, No. 11, pp. 3942–3946, November 1976
Biochemistry

Isolation, primary structure, and synthesis of α-endorphin and γ-endorphin, two peptides of hypothalamic-hypophysial origin with morphinomimetic activity

(opiates/endogenous ligand/mass spectrometry/high pressure chromatography)

NICHOLAS LING, ROGER BURGUS, AND ROGER GUILLEMIN

The Salk Institute, La Jolla, California 92037

Contributed by Roger Guillemin, August 30, 1976

ABSTRACT The isolation and primary structure of two peptides with morphinomimetic activity, obtained from an extract of porcine hypothalamus-neurohypophysis, are described. The amino acid sequence of the two peptides, named α-endorphin and γ-endorphin, was determined by mass spectrometry and dansyl-Edman methods to be H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH and H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OH, respectively. These correspond to the amino acid sequences present between residues 61 and 76 and residues 61 and 77 of the various β-lipotropins. A third peptide also obtained in pure form in these studies was found to be an unstable salt of α-endorphin.

In a preliminary note (1) we have reported isolating from a crude extract of (porcine) hypothalamus-neurohypophysis three peptides named *endorphins* with morphine-like activity in a bioassay and in a synaptosomal opiate-receptor binding assay. In that same note we presented the primary structure of one of these peptides, *α-endorphin,* to be that of the hexadecapeptide H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH. The present communication will describe in detail: (*i*) the starting material utilized, (*ii*) the method of isolation of these peptides, (*iii*) the methods used to establish the amino acid sequence of both α-endorphin and γ-endorphin, and (*iv*) evidence that the third isolated peptide is, in fact, an unstable salt of α-endorphin.

MATERIALS AND METHODS

Assay of Biological Activity. It was decided that morphinomimetic substances would be defined as those substances which would decrease linearly the amplitude of the muscle contractions electrically induced *in vitro* in the myenteric plexus-longitudinal muscle of the guinea pig ileum (2) only when this biological activity would be reversed or prevented by naloxone, a morphine-analogue antagonist.

Starting Material. We first confirmed reports by others (3–5) that aqueous extracts of whole brain or of several specific anatomical brain structures (caudate nucleus, hypothalamus) or of the pituitary gland (6) did contain naloxone-reversible morphinomimetic activity. Calculations based on simple assumptions relating expected specific activity of the substances to be characterized to their apparent concentration in these extracts indicated that several hundreds of kg of fresh tissues would have to be procured and handled to provide a reasonable chance of characterizing the postulated substances again within a reasonable time schedule. Searching through materials available to us in large quantities from our earlier isolation program of the hypothalamic-hypophysiotropic factors, we found a partially purified extract of (porcine) neurohypophysis-hypothalamus to be already considerably enriched in morphinomimetic activity (half maximal activity in the bioassay at about 10 μg of the dry powder per ml of incubation fluid). This material was used for the isolation of the endorphins; it is an acetic acid-acetone extract corresponding to fraction G of the Kamm procedure (7) performed on tissues of porcine origin consisting of approximately 50% neurohypophysis and 50% pituitary stalk and attached ventral hypothalamus (Pitressin Intermediate, Parke-Davis and Co.). Assays performed in our laboratories in the Fall of 1975 showed this material to contain 12 USP units of vasopressin per mg, 4 USP units of oxytocin per mg, 2×10^5 Shizume units of melanotropin (MSH) activity per mg, 1.67 international units of adrenocorticotropin (ACTH) per mg by an *in vitro* corticoidogenic assay. One hundred grams of this material, corresponding to about 1/4 million posterior pituitary-hypothalamus, were used in the purification described below. (See Note Added in Proof.)

Purification Scheme. We accepted earlier evidence in the literature (3–5) that the morphinomimetic substances of brain origin were polypeptides. The purification sequence finally established from pilot studies consisted of five steps (*i* to *v*) as shown below.

(*i*) *Gel filtration.* Two aliquots of the powder, 50 g and 40 g, were dissolved at 4° in 2 M HOAc (100 mg of powder per ml) by constant stirring for 2 hr. Insoluble material was removed by centrifugation. Each aliquot was successively sieved on Sephadex G-25 (10 × 250 cm, V_{bed} = 16.7 liters, V_0 = 6.5 liters). Filtration was performed by gravity at a flow rate of 600 ml/hr; 250-ml fractions were collected. Practically all the naloxone-reversible morphinomimetic activity is located in a narrow zone at V_e/V_0 = 1.4 (pool 2; total yield 8.91 g).

(*ii*) *SP-Sephadex chromatography.* Material of pool 2-(*i*) was dissolved in 100 ml of 0.001 M NH_4OAc, pH 4, and then applied to a SP-Sephadex C-25 column (5 × 40 cm, V_{bed} = 800 ml) equilibrated in 0.001 M NH_4OAc, pH 4. Elution of the peptides was performed stepwise with 2.8 liters of 0.001 M NH_4OAc, pH 4; 4.3 liters of 0.01 M NH_4OAc, pH 7; and 4.5 liters of 2 M NH_4OAc, pH 7. The results obtained were not in agreement with those of several pilot studies on mg amounts, in which the biological activity was sharply separated. In the preparative column, the biological activity eluted broadly with 0.01 M to 2 M NH_4OAc; these fractions were pooled and lyophilized (4.25 g), with only a minor gain in purification.

(*iii*) *DEAE-Sephadex chromatography.* The lyophilized powder from (*ii*) was dissolved in 350 ml of 0.001 M NH_4OAc, pH 7, and the solution applied on a DEAE-Sephadex A-25 column (6 × 21 cm, V_{bed} = 600 ml) equilibrated in 0.001 M NH_4OAc, pH 7. Elution was conducted stepwise with 6.8 liters of 0.001 M, 7.2 liters of 0.01 M, 2.3 liters of 0.02 M, 2.2 liters of

Abbreviations: HPLC, high pressure liquid chromatography; LPH, lipotropin.

0.05 M, 8.5 liters of 0.1 M NH_4OAc, all at pH 7. Fractions of 100 ml were collected. Two zones of biological activity eluted between fractions no. 1 and 20 (pool 1) and 164 and 210 (pool 3) were localized. Quantitative 4-point bioassays with factorial analysis of the results showed that pool 3 contained 2.6 times more units of biological activity than pool 1; however, the specific activity of the material contained in pool 1 was five times greater than that of pool 3. It was thus decided to purify further the material (560 mg) present in pool 1. Subsequent rechromatography of an aliquot of either pool (1 or 3) on the same exchanger, under the same conditions, led to the appearance of two peaks corresponding to the original pool 1 and pool 3. With subsequent knowledge of the primary structure of the major components of pool 1 (see below), this observation is best explained by assuming that the materials in pool 1 and pool 3 are identical but of different conformations or degrees of association so that a negative charge is exposed in the case of materials retarded on the ion exchanger, as in pool 3.

(*iv*) *Partition chromatography.* A portion of pool 1-(*iii*) material (457 mg) was applied to a Sephadex G-50 fine partition column (2.5 × 100 cm, V_{bed} = 490 ml, V_0 = 164 ml) and eluted with the solvent system 1-BuOH:HOAc:H_2O (4:1:5). Three major zones of biological activity were located: pool 4, R_F = 0.51, 23.0 mg; pool 6, R_F = 0.30, 24.8 mg; and pool 9, R_F = 0.20, 32.2 mg.

(*v*) *High pressure liquid chromatography* (*HPLC*). Conditions for HPLC that led to the pure products will be described under *Results*. The apparatus for HPLC is the Waters Associates model 204 system. An Infotronics model 110 integrator was used to determine peak areas.

Amino Acid Analysis. Amino acid composition of peptides was determined as previously described (8) after hydrolysis in 6 M HCl–0.5% thioglycollic acid or enzymatic digestion with papain and leucineaminopeptidase (9).

Edman Degradation. Sequential degradation of peptides was performed using the Edman method followed by dansylation with [^{14}C]dansyl chloride and identification of Pth-derivatives by mass spectrometry as previously described (8, 10).

Mass Spectrometry. As will be shown below, three substances were obtained in pure form. They will be referred to here as α-endorphin-1, α-endorphin-2, and γ-endorphin. α-Endorphin-1 (175 μg, 100 nmol) was digested with 1.75 μg of trypsin (Worthington) in 100 μl of 0.1 M NH_4OAc–10^{-3} M $CaCl_2$ at pH 8.1 for 2 hr at 37°. The digestion was terminated with 2 drops of glacial HOAc and the solution was lyophilized twice. Acetylation of the resulting peptide fragments was accomplished by heating the residue with 20 μl of a 50:50 (vol/vol) mixture of $(CD_3CO)_2O$ and $(CH_3CO)_2O$ in 100 μl of HOAc at 100° for 2 min. The solvent was evaporated *in vacuo* and the residue relyophilized from 100 μl of H_2O. Permethylation of the acetylated peptide fragments was performed by a procedure published previously (11) with the following exceptions: the strength of the $NaCH_2SOCH_3$ base was determined by 0.01 M HCl titration, and a 20 times excess of the base over the total equivalent of all the replaceable hydrogens plus a molar amount of CH_3I equal to the base were used. Mass spectra were obtained from a Varian Mat CH-5 single-focusing mass spectrometer using the same conditions as in the original description of the method (11).

An identical procedure was used to obtain the amino acid sequence of α-endorphin-2 and γ-endorphin.

Synthesis. α-Endorphin and γ-endorphin were prepared by solid phase methodology, purified and tested for homogeneity by the methods routinely used in this laboratory (12, 13).

Table 1. Amino acid analyses of native and synthetic porcine endorphins

	Native porcine endorphin				Synthetic endorphin		
	α-1[a]	α-2[a]	α-2[b]	γ[a]	α[b]	α[b]	γ[a]
Thr	2.7	2.6	2.4	2.7	2.9	2.0	2.8
Gln	—	—	—	—	—	—	—
Ser	1.6	1.5	1.9[c]	1.6	1.7	1.6[c]	1.8
Glu	2.0	2.0	1.0	1.9	2.0	0.9	2.0
Pro	1.0	1.0	—	1.0	1.0	—	0.9
Gly	1.9	1.9	1.9	2.0	2.1	2.0	2.0
Val	1.0	1.0	1.1[d]	1.0	1.0	1.2[d]	1.0
Met	1.0	1.0	0.9	0.9	1.0	1.0	1.0
Leu	1.1	1.0	1.3[d]	2.0	1.0	1.4[d]	2.0
Tyr	1.0	1.0	0.9	0.9	1.0	1.1	1.0
Phe	1.0	1.0	0.9	1.0	1.0	1.1	1.0
Lys	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NH_3	2.3	1.6	—	2.3	1.2	—	1.3

[a] Hydrolysis in 6 M HCl-0.5% thioglycollic acid.
[b] Enzymatic hydrolysis with papain and leucineaminopeptidase.
[c] Gln does not resolve from Ser under these conditions.
[d] This peak includes a shoulder, probably due to incompletely hydrolyzed peptides.

RESULTS

α-Endorphin-1. A 1.5-mg sample of pool 6-(*iv*) was further purified by HPLC on a 4 mm × 30 cm μ-Bondapak/C18 column using solvent concentration gradient curve 8 computed by a Waters model 660 programmer. Starting buffer A was 20% CH_3CN in 0.01 M NH_4OAc, pH 4, and final buffer B was CH_3CN at a program rate of 0–80% B during 20 min. The flow rate was 2.5 ml/min. The biologically active zone (366 μg) eluted between 9.2 and 11.2 min, and a sharp cut was essentially homogeneous, as indicated by analytical HPLC. Hydrolysis by HCl yielded the amino acid ratios shown in Table 1. A sample of this material, now named α-endorphin-1, was subjected to trypsin digestion followed by acetylation and permethylation for direct sequence determination by mass spectrometry. As the temperature of the direct-inlet probe containing the derivatized peptide was gradually raised over 200°, a doublet at m/e 158 and 161 began to appear in the mass spectrum, indicating a NH_2-terminal Ser for one of the cleaved peptide fragments. Further raising of the temperature from 231 to 245° revealed a series of doublet peaks corresponding to a peptide with the sequence H-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH (see Fig. 1). This sequence is substantiated by another series of doublets resulting from the loss of CH_3OH from each of the corresponding sequence ions due to the presence of Ser and Thr. That m/e 954 and 957 are molecular ions of this peptide was confirmed by the doublet at m/e 939 and 942, which corresponds to the loss of a methyl group. Since the COOH-terminus of this peptide fragment ends as a Thr-COOH and α-endorphin-1 contains only one Lys (Table 1), it is logical to place this fragment at the COOH-terminus of α-endorphin-1.

Further elevation of the probe temperature from 280 to 295° revealed the incomplete sequence of another peptide fragment as H-Tyr-Gly-Gly-Phe-Met-Thr-Ser- (see Fig. 2). This sequence is confirmed by a series of singlet peaks corresponding to the loss of *N*-methyl acetamide from the NH_2-terminus of each sequence ion through a McLafferty type of rearrangement (14). Since no ions with m/e higher than 942 were detected, the complete sequence of this fragment cannot be ascertained. However, from the already established COOH-terminal fragment H-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH and the partial

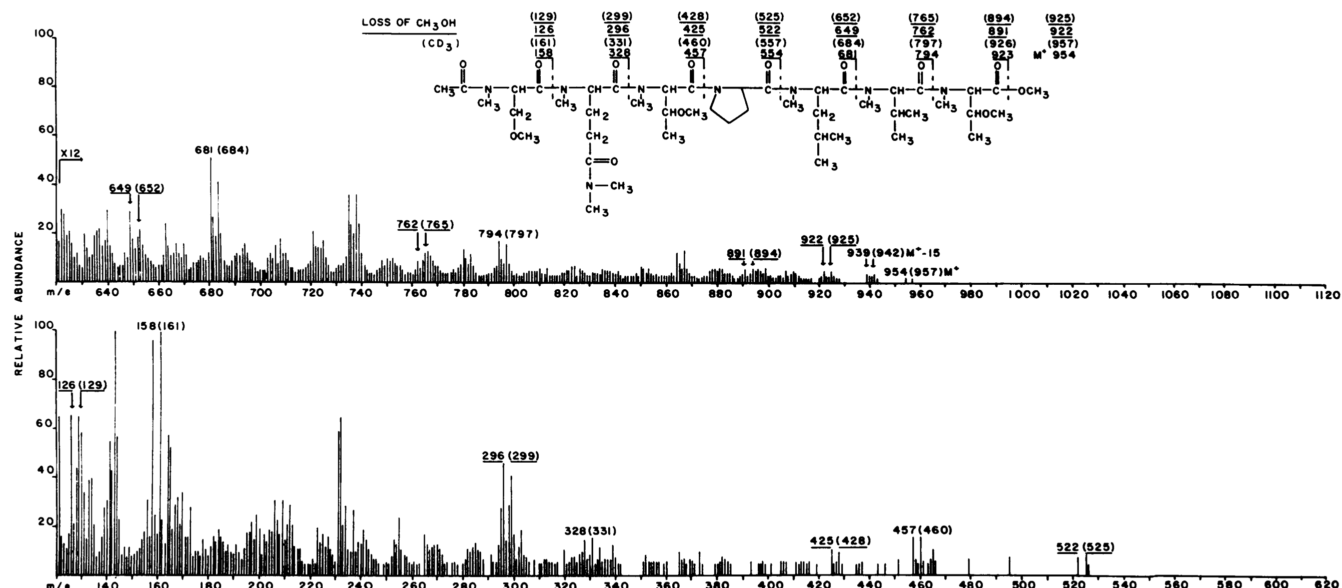

FIG. 1. Mass spectrum of derivatized α-endorphin-1 obtained by fractional vaporization at 231–245°. The peptide detected has the sequence H-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH.

sequence H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-, all the amino acids of α-endorphin-1, with the exception of one Lys and one Glu or Gln, have been accounted for. As a result, we can write the tentative primary structure of α-endorphin-1 as H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glx-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH, bearing in mind that the molecule has only one Lys.

While the mass spectrometric analysis was in progress, a 284 μg (163 nmol) sample of the same material was subjected to Edman degradation with the results shown in Table 2. Coupling in the third cycle was apparently incomplete since there appeared to be a mixture of two peptides (one being longer than the other by one residue) being sequenced in the subsequent cycles. Nevertheless, the results were consistent with the amino acid sequence determined by mass spectrometry. Furthermore, mass spectrometric analysis of the Pth-derivatives from the seventh and eighth Edman cycles showed them to be Pth-Glu and (ε-Ptc)-Pth-Lys, respectively, thus establishing the complete sequence of porcine α-endorphin-1 as H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH.

α-Endorphin-2. HPLC purification of 1.5 mg of pool 9-(*iv*) was carried out using conditions similar to those described above for α-endorphin-1, except that the starting buffer A was 23% CH_3CN and the gradient was 0–100% B. Under those conditions the biologically active zone (478 mg) occurred in a peak with retention time at 7.8 min; α-endorphin-1 eluted at 4.5 min in this system. Amino acid composition after HCl and enzymatic hydrolysis (Table 1) and sequence determination by mass spectrometry showed this compound (then named α-endorphin-2) to be identical to α-endorphin-1. Therefore, the only

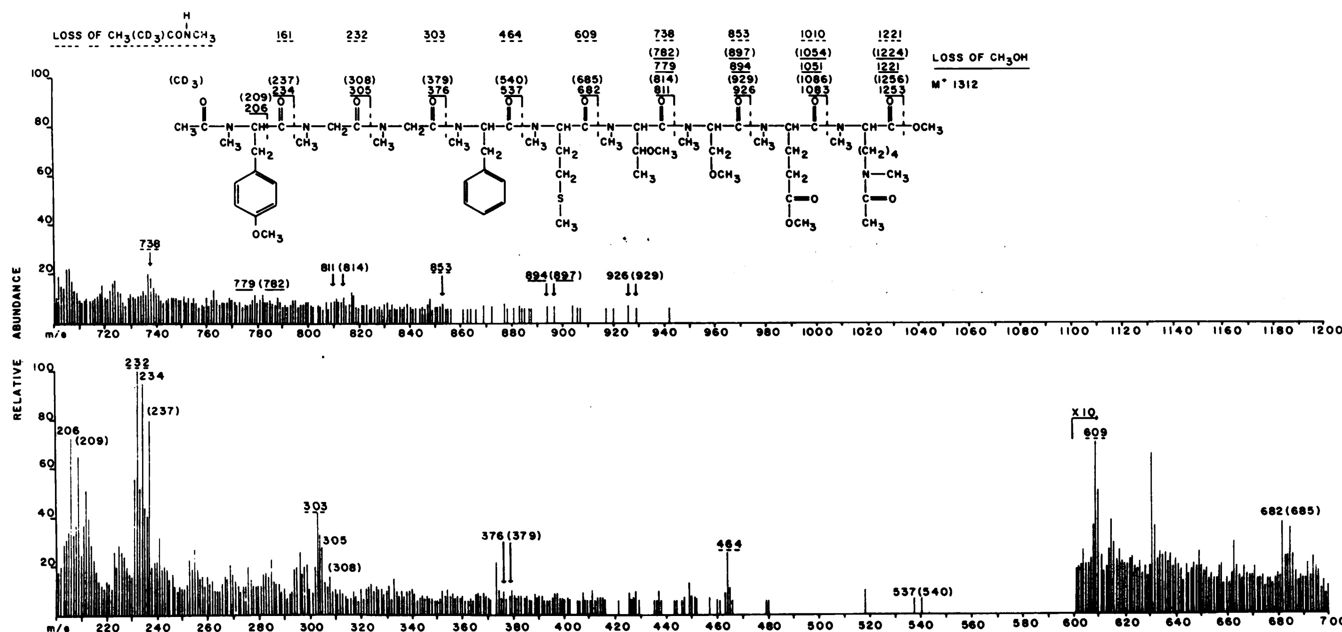

FIG. 2. Mass spectrum of derivatized α-endorphin-1 obtained by fractional vaporization at 280–295°. The peptide detected has the sequence H-Tyr-Gly-Gly-Gly-Phe-Met-Thr-Ser-.

Table 2. Dansyl derivatives from Edman degradation of porcine α-endorphin (net cpm/Edman cycle)

Dns-amino acid	Edman cycle 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Control	n*
Tyr/2	*23,921*	0	0	0	301	194	0	66	549	0	13	0	0	240	0	293	1912 ± 571	15
Gly	0	*5,170*	*10,239*	0	0	795	0	0	0	0	0	0	1,991	3,424	206	0	1508 ± 1344	14
Phe	882	590	624	*2,253*	1,303	0	0	0	1,744	0	578	0	0	555	0	0	855 ± 825	14
Met	1	95	61	0	*5,691*	1,771	215	15	0	0	0	0	0	525	0	0	503 ± 100	14
Thr	0	0	0	0	0	*4,987*	1,625	1,118	90	351	62	*4,518*	2,919	2,738	20	*3,121*	1428 ± 1231	10
Ser	0	0	0	0	0	0	*4,695*	3,383	834	*3,590*	1,816	70	889	0	560	12	760 ± 503	12
Glx	0	0	0	0	0	16	0	*12,634*	7,451	1,758	*8,151*	2,603	1,146	1,530	0	0	1046 ± 963	12
Ptc-Lys	—	—	—	—	—	—	—	—	*2,502*	1,038	1,040	0	0	—	160	648	1310 ± 834	6
Pro	0	78	382	364	244	224	0	0	0	0	0	0	*3,814*	716	0	0	604 ± 200	13
Leu	201	0	0	0	0	0	268	695	412	624	748	302	0	*8,298*	1,760	0	1454 ± 546	13
Val	0	0	0	0	0	336	0	7	0	0	0	0	107	1,835	*5,136*	0	1109 ± 543	14
nmol used	1	1	1	1	2	2	2	3	3	3	3	3	4	4	4	4		

Italicized values are those judged to represent newly formed terminal amino acids.
* n equals the number of determinations of the control values.

physical property that distinguishes this compound from α-endorphin-1 is that it migrates more slowly in partition chromatography as well as in HPLC. We observed, however, that after letting the lyophilized α-endorphin-2 stand at room temperature for a few hours, the resulting material showed the same retention time as α-endorphin-1 when it was reinjected into the HPLC column. This observation is best explained by proposing that α-endorphin-2 is probably an unstable salt form of α-endorphin-1. Similar results have been observed and a similar explanation proposed by Noble *et al.* (15) in studies dealing with synthetic fragments of prolactin. We will thus refer to the single peptide component as α-endorphin.

γ-Endorphin. HPLC purification (60 cm μ-Bondapak/C 18, 25% CH_3CN, 2.5 ml/min) of 800 μg of pool 4-(*iv*) yielded a homogeneous active fraction (172 μg) with retention time between 10.0 and 11.2 min. Amino acid determination of a HCl hydrolyzate gave the ratios shown in Table 1. This material was named γ-endorphin because, in the intervening time, after we had characterized α-endorphin, C. H. Li proposed the name β-endorphin for the C-fragment of β-lipotropin (available from previous chemical studies) when it was shown to possess morphinomimetic activity (see below, *Discussion*).

A sample (100 nmol) of γ-endorphin was subjected to the identical derivatization procedure as α-endorphin for direct mass spectrometric determination of its sequence. When the direct-inlet probe temperature was raised from 237 to 246°, a series of sequence ions corresponding to a peptide with the sequence H-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OH was obtained (see Fig. 3). Further elevation of the temperature from 296 to 313° yielded a mass spectrum practically identical to that shown in Fig. 2. Using the same reasoning as above, the tentative primary structure for γ-endorphin can be written as H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glx-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OH.

Another 186 μg (100 nmol) of this material was treated with 4.6 μg of α-chymotrypsin in 100 μl of 0.1 M NH_4OAc, pH 8.1, for 5 hr at 37°. Sequencing by the dansyl-Edman method of the digested mixture and mass spectrometric analysis of the resulting Pth-derivatives gave data that were best interpreted as corresponding to a mixture of five peptide fragments: H-Tyr-Gly-Gly-Phe-OH, H-Tyr-Gly-Gly-Phe-Met-OH, H-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-OH, H-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-OH, and H-Val-Thr-Leu-OH. These results, together with the mass spectrometric data, established the sequence H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OH for porcine γ-endorphin.

Both α-endorphin and γ-endorphin were synthesized by solid

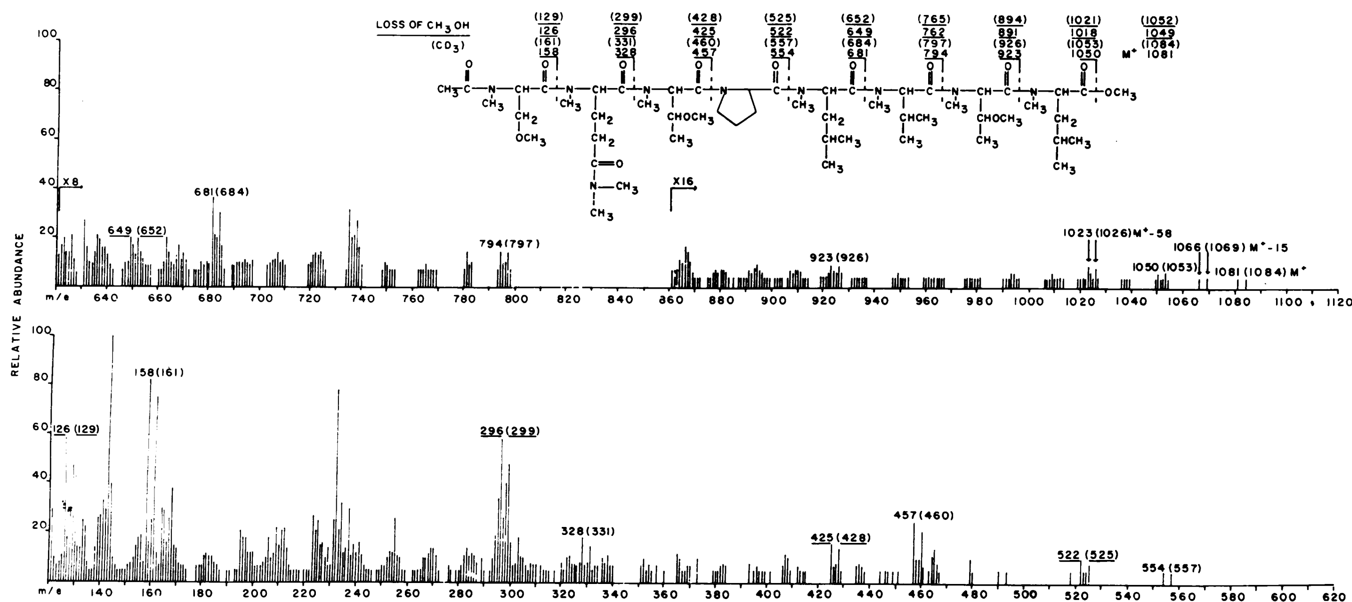

FIG. 3. Mass spectrum of derivatized γ-endorphin obtained by fractional vaporization at 237–246°. The peptide detected has the sequence H-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OH.

phase methodology (16). The synthetic materials showed the same amino acid compositions (Table 1), HPLC patterns, R_F values on thin-layer chromatography [Eastman 13191 silica gel sheet with solvent system 1-BuOH:HOAc:H_2O (4:1:5), upper phase], mass spectra (Figs. 1–3), and biological activity as the respective native substance.

DISCUSSION

As this work was in progress, Hughes *et al.* reported (17) that the characterization of porcine *enkephalin*, which had been purified earlier (3), showed it to be in fact the two pentapeptides H-Tyr-Gly-Gly-Phe-Met-OH and H-Tyr-Gly-Gly-Phe-Leu-OH. They also, in the same report, made the remarkable observation that the primary structure of Met^5-enkephalin was identical to the sequence Tyr 61-Met 65 of the hypophysial polypeptide β-lipotropin (β-LPH) isolated in 1964 by C. H. Li and collaborators (reviews of references in ref. 18), while Leu^5-enkephalin would share with β-LPH the sequence Tyr 61-Phe 64. α-Endorphin and γ-endorphin have, respectively, for primary structure, the same amino acid sequence as β-LPH-(61-76) and β-LPH-(61-77). The C-fragment of β-LPH [β-LPH-(61-91)] has been shown to have also morphinomimetic activity (19–23), and C. H. Li has named it β-endorphin (23). A number of papers have already appeared speculating on the new and unexpected possible physiological role of β-LPH as a prohormone for the endorphins and enkephalins. Recent reports on the profound behavioral effects of the endorphins, all reversible by naloxone, have led to more speculations about their possible role in the pathogenesis of certain mental diseases (24). Characterization of these peptides, apparently present normally in the central nervous system, remains the *sine qua non* for all the present and future physiological and clinical developments.

Note Added in Proof. In collaboration with Floyd Bloom, immunocytochemical studies with highly specific antisera raised against α-endorphin and β-endorphin have recently demonstrated that these two peptides are present in cells of the intermediate and anterior lobes of the pituitary gland of the rat; they are not found in the posterior lobe of the pituitary (neurohypophysis). They are also seen in nerve fibers in the median eminence and the hypothalamus. Maurice Dubois (personal communication) has made similar observations with antisera of his own elicited against α-endorphin, using pituitary and hypothalamic tissues of bovine and porcine origin. Presence of the endorphins in the crude extract (Pitressin Intermediate) described here as of neurohypophysial origin is explained by realizing that tissues of the intermediate lobe always remain attached to the posterior lobe as it is dissected away from the whole pituitary gland (note the high concentration of biological melanophoretic activity present in that extract—about 1/10 of the specific activity of pure α-melanotropin, as reported above in *Materials and Methods*).

We would like to recognize the excellent technical help of Scott Minick, Madalyn Butcher, Maluz Mercado, Larry Koski, and Roland Schroeder. Research supported in part by the W. R. Hearst Foundation, the National Institutes of Health (NICHD 8852).

1. Guillemin, R., Ling, N. & Burgus, R. (1976) *C. R. Hebd. Seances Acad. Sci. Ser. D* **283**, 783–785.
2. Paton, D. M. & Zar, M. A. (1968) *J. Physiol. (London)* **194**, 13–33.
3. Hughes, J., Smith, T., Morgan, B. & Fothergill, L. (1975) *Life Sci.* **16**, 1753–1758.
4. Pasternak, G. W., Goodman, R. & Snyder, S. H. (1975) *Life Sci.* **16**, 1765–1768.
5. Terenius, L. & Wahlstrom, A. (1974) *Acta Pharmacol. Toxicol.* **35**, 55–58.
6. Cox, B. M., Opheim, K. E., Teschemacher, H. & Goldstein, A. (1975) *Life Sci.* **16**, 1777–1782.
7. Kamm, O., Aldrich, T. B., Grose, W., Rowe, L. W. & Bugbee, E. P. (1928) *J. Am. Chem. Soc.* **50**, 573–601.
8. Burgus, R., Ling, N., Butcher, M. & Guillemin, R. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 684–688.
9. Stewart, J. & Young, J. (1969) *Solid Phase Peptide Synthesis*, (W. H. Freeman, San Francisco), pp. 54–55.
10. Burgus, R., Butcher, M., Amoss, M., Ling, N., Monahan, M., Rivier, J., Fellows, R., Blackwell, R., Vale, W. & Guillemin, R. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 278–282.
11. Ling, N., Rivier, J., Burgus, R. & Guillemin, R. (1973) *Biochemistry* **12**, 5305–5310.
12. Ling, N. & Vale, W. (1975) *Biochem. Biophys. Res. Commun.* **63**, 801–806.
13. Ling, N., Rivier, J., Monahan, M. & Vale, W. (1976) *J. Med. Chem.* **19**, 937–941.
14. Grützmacher, H.-F. & Heyns, K. (1966) *Justus Liebigs Ann. Chem.* **698**, 24–33.
15. Noble, R., Yamashiro, D. & Li, C. H. (1976) *J. Am. Chem. Soc.* **98**, 2324–2328.
16. Merrifield, R. B. (1963) *J. Am. Chem. Soc.* **85**, 2149–2154.
17. Hughes, J., Smith, T., Kosterlitz, H., Fothergill, L., Morgan, B. & Morris, H. (1975) *Nature* **258**, 577–579.
18. Yamashiro, D. & Li, C. H. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4945–4949.
19. Bradbury, A., Smyth, D., Snell, C., Birdsall, N. & Hulme, E. (1976) *Nature* **260**, 793–795.
20. Cox, B., Goldstein, A. & Li, C. H. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1821–1823.
21. Lazarus, L., Ling, N. & Guillemin, R. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2156–2159.
22. Ling, N. & Guillemin, R. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3308–3310.
23. Li, C. H. & Chung, D. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1145–1148.
24. Bloom, F., Segal, D., Ling, N. & Guillemin, R. (1976) *Science* **194**, in press.