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Common precursor to corticotropins and endorphins

(pituitary tumor cells/β-lipotropin/β-melanotropin/peptide analysis/immunoprecipitation)

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ABSTRACT **Double-antibody immunoprecipitation procedures with antisera to endorphins and to corticotropin (ACTH) were used to study the biosynthesis of these peptides in a mouse pituitary tumor cell line. Cultures were incubated with a ^{3}H-labeled amino acid, and aliquots of culture medium were immunoprecipitated. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis of [^{3}H]phenylalanine-labeled immunoprecipitates prepared with endorphin antisera resolved three forms of endorphin with apparent molecular weights of 31,000, 11,700, and 3500; immunoprecipitates prepared with the ACTH antiserum contained four forms of ACTH with apparent molecular weights of 31,000, 23,000, 13,000, and <4500. Sequential immunoprecipitation of culture medium with the ACTH antiserum and then with the endorphin antiserum (or the reverse order) indicated that both antisera precipitated the same 31,000 dalton molecule. Purified pools of the different forms of ACTH and endorphin were prepared by immunoprecipitation and gel filtration. The tryptic peptides found in [^{3}H]phenylalanine- or [^{3}H]tryptophan-labeled 31,000 dalton ACTH were identical to the tryptic peptides found in digests of 31,000 dalton endorphin labeled with the same amino acid. A tryptic peptide similar to the lipotropin tryptic peptide [βLPH(61-69)] that contains the opiate-active methionine-enkephalin sequence could be identified in 31,000 dalton ACTH and in all the different forms of endorphin. Most of the peptide cleaved from 31,000 dalton ACTH when it is converted to 23,000 dalton ACTH could be precipitated by endorphin antisera; this 11,700 dalton endorphin molecule is similar to the pituitary hormone βLPH in size and structure. The 3500 dalton endorphin is similar to β-endorphin in size and structure. The culture medium from the AtT-20 mouse pituitary tumor cells contained approximately equimolar amounts of ACTH-related peptides and endorphin-related peptides.**

The pituitary hormone β-lipotropin (βLPH) was first purified and sequenced more than a decade ago (1). A physiological role for βLPH as either a lipolytic hormone or as a precursor of β-melanotropin (βMSH) has never been convincingly demonstrated (2, 3). The recent discovery that fragments of the COOH-terminal region of βLPH have potent opiate activity (4–7) has generated renewed interest in the synthesis and secretion of βLPH. Several different observations suggest a close relationship among βLPH, endorphins, and ACTH: (*i*) under most physiological and pathological conditions, stimuli that alter ACTH release from the pituitary alter βLPH release in a parallel manner (8, 9); (*ii*) ectopic ACTH-secreting tumors usually secrete peptides with βMSH immunoreactivity (10, 11); (*iii*) immunochemical studies have shown that βLPH, endorphins, and ACTH occur in the same pituitary cells (12–15) and within the same secretory granules (12); and (*iv*) studies on human pituitary extracts suggest that antigenic determinants for ACTH and LPH occur in the same molecule (16).

Abbreviations: βLPH, β-lipotropin; βMSH, β-melanotropin; NaDodSO_4, sodium dodecyl sulfate; Gdn, guanidine; the abbreviations used for the cell products studied are based on two characteristics—apparent molecular weight in the borate-acetate buffered NaDodSO_4/polyacrylamide gel electrophoresis system (31k, 23k, etc.) and the antiserum used to immunoprecipitate the molecule (ACTH or endorphin).

The ACTH-secreting mouse pituitary tumor cell line AtT-20/D-16v has been used to study ACTH synthesis (17–19). These tumor cells, as well as normal pituitary tissue, contain several high-molecular-weight glycoprotein forms of ACTH with molecular weights of 31,000, 23,000, and 13,000 (designated 31k ACTH, 23k ACTH, and 13k ACTH, respectively). The largest, 31k ACTH, is the biosynthetic precursor of the smaller forms of ACTH, and 23k ACTH is a biosynthetic intermediate. In the course of structural studies on 31k ACTH and 23k ACTH labeled with ^{3}H-labeled amino acids, analysis of tryptic peptides suggested that the fragment cleaved off during the conversion of 31k ACTH to 23k ACTH might be similar to βLPH. In the experiments presented here, immunoprecipitation of ^{3}H-labeled cell products with well-characterized antisera to both ACTH and endorphin and peptide analysis were used to demonstrate that 31k ACTH contains peptides similar to both ACTH α(1-39) and β-endorphin.

METHODS

Incubation of Cells with ^{3}H-Labeled Amino Acids and Immunoprecipitations. AtT-20/D-16v cells were grown in microtest wells and incubated with a ^{3}H-labeled amino acid in Dulbecco-Vogt modified Eagle's medium containing 2.5% horse serum for 8 hr as described (17, 18). Under the incubation conditions used (see legend to Fig. 1), incorporation of labeled amino acid into trichloroacetic acid-precipitable material never exceeded 8% of the total amount of labeled amino acid present; under these conditions, phenylalanine and tryptophan are not converted to other amino acids. Media and cells were harvested with protease inhibitors (18).

Double-antibody immunoprecipitations of culture medium containing ^{3}H-labeled cell products were performed with several different specific antisera. Purified ACTH α(1-24) antiserum was prepared as described (18). Rabbit antisera to synthetic α- and β-endorphin (14, 20) were generously supplied by R. Guillemin (Salk Institite, CA). The amount of β-endorphin antiserum necessary for quantitative immunoprecipitation of material from samples of culture medium was determined by measuring the effect of added culture medium on the binding of ^{125}I-labeled β-endorphin to the β-endorphin antiserum (18). A sample of ^{3}H-labeled culture medium was incubated with specific antiserum at 4° for 16 hr and an immunoprecipitate was formed by addition of goat antiserum to rabbit immunoglobulin (Pacific Biologicals). All buffers used to prepare immunoprecipitates were identical to those described previously, except that buffer E contained 1% Triton X-100 (18). The specificity of immunoprecipitates prepared with endorphin antisera was demonstrated by comparing immunoprecipitates prepared in the presence and absence of excess unlabeled synthetic peptide.

Analysis of ^{3}H-Labeled Immunoprecipitates. Immunoprecipitates were analyzed by sodium dodecyl sulfate (NaDodSO_4)/polyacrylamide gel electrophoresis (borate-acetate

buffered, pH 8.5) as described (18, 21). Dansylated cytochrome *c* was used as an internal standard in all gels. Pools containing a single molecular weight form of ACTH or endorphin were prepared by gel filtration of ^{3}H-labeled immunoprecipitates on columns of Sephadex G-75 equilibrated with 10% (vol/vol) formic acid containing bovine serum albumin (20 μg/ml). The separated pools of ^{3}H-labeled endorphin were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis in a phosphate-buffered system (22) or by gel filtration on Sephadex G-50 in 6 M guanidine hydrochloride (Gdn·HCl) (19).

Analysis of Tryptic Digests. Pools of ^{3}H-labeled ACTH or endorphin were dissolved in 100 μl of 0.2 M NH_4HCO_3 containing 100 μg of bovine serum albumin. TPCK-trypsin (Worthington) was added in a weight ratio of 1:20 and digestion was allowed to proceed for 5–6 hr at 37°; a second addition of trypsin was made and the incubation was stopped 9–11 hr later. Peptides were separated by electrophoresis on Whatman 3MM paper at 800 V/50 cm for 2–3 hr; two pyridine/acetic acid/water buffers were used—pH 6.35 (25:1:225) and pH 3.5 (1:10:189). Lysine and dinitrophenyllysine or glycine were included in all samples as internal standards and were visualized with ninhydrin; peptide mobilities were calculated by defining the center of dinitrophenyllysine as 0 and the center of lysine as 1.00. Peptides were analyzed by descending paper chromatography in *n*-butanol/acetic acid/water (4:1:5; upper phase); phenylalanine was included as an internal standard in all chromatographic separations (its mobility was defined as 1.00). Paper strips were cut into 1-cm sections and ^{3}H-labeled peptides were eluted into 0.5 ml of 0.1 M HCl for at least 2 hr; recovery of radioactivity was 80–100%.

RESULTS

Immunoprecipitation. In order to test the hypothesis that 31k ACTH contains a βLPH- or endorphin-like sequence in addition to the α(1-39) sequence, AtT-20 cells were incubated with [^{3}H]phenylalanine and separate aliquots of culture medium were analyzed by immunoprecipitation with antisera to ACTH α(1-24) and to β-endorphin (Fig. 1 *upper*). Four forms of ACTH could be detected after immunoprecipitation with purified ACTH α(1-24) antiserum: molecular weights 31,000, 23,000, 13,000, and <4500. Analysis of a β-endorphin immunoprecipitate in the same gel electrophoresis system demonstrated the presence of three peaks of material with apparent molecular weights of 31,000, 11,700, and 3500. The β-endorphin antiserum and the ACTH α(1-24) antiserum precipitated the same amount of 31k material. β-Endorphin immunoprecipitates of culture medium containing [^{3}H]tryptophan-labeled cell products contained only two peaks of immunoprecipitable material: 31k endorphin and 11.7 k endorphin (Fig. 1 *lower*). Fig. 1 *lower* demonstrates that the β-endorphin immunoprecipitation scheme is specific. Additional experiments with two antisera to synthetic α-endorphin gave results similar to those shown in Fig. 1.

The 31k molecule was the only species that appeared to have determinants recognized by both the ACTH α(1-24) antibody and the β-endorphin antibody (Fig. 1). If the two antisera precipitate the same 31k molecule, it should be possible to demonstrate the identity by using the two antisera sequentially. The ACTH α(1-24) antiserum was used to remove all ACTH α(1-24)-related molecules from a sample of [^{3}H]phenylalanine-labeled culture medium; the supernatant from this immunoprecipitate was then analyzed by immunoprecipitation with the β-endorphin antibody (Fig. 2 *upper*). The initial immunoprecipitation with ACTH α(1-24) antiserum completely eliminated the 31k molecule from the subsequent β-endorphin

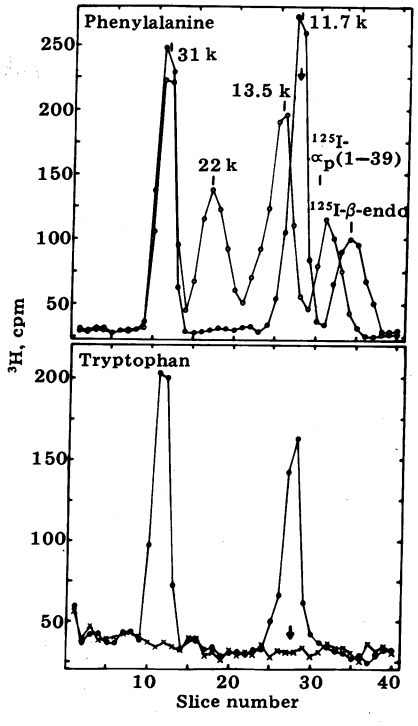

FIG. 1. Immunoprecipitation of AtT-20 culture medium with antisera to ACTH and endorphin, followed by $NaDodSO_4$/polyacrylamide gel electrophoresis. (*Upper*) Phenylalanine as label. A confluent microwell of AtT-20 cells was incubated for 8 hr in 100 μl of phenylalanine-deficient medium containing 150 μM L-phenyl[2,3-^{3}H(N)]alanine (Research Products Inc.; 10 Ci/mmol). Aliquots (10 μl) of medium were precipitated with either affinity-purified ACTH α(1-24) antiserum or β-endorphin antiserum. The immunoprecipitates were analyzed by borate-acetate buffered $NaDodSO_4$/polyacrylamide gel electrophoresis (18). Dansylated cytochrome *c* (arrow) was included in each gel sample; plots have been adjusted to make the dansylated cytochrome *c* markers coincident. ^{125}I-Labeled ACTH α_p(1-39) and ^{125}I-labeled β-endorphin were analyzed on parallel gels. ●, β-Endorphin antiserum (RB100), cpm/slice; ○, purified ACTH α(1-24) antiserum (Bertha), cpm/slice. (*Lower*) Tryptophan as label. A confluent microwell of AtT-20 cells was incubated for 8 hr in 100 μl of tryptophan-deficient medium containing 40 μM L-[*G*-^{3}H]tryptophan (Amersham/Searle; 7 Ci/mmol). A 30-μl aliquot of medium was immunoprecipitated with β-endorphin antiserum; excess (5 μg) synthetic β-endorphin was added to another 30-μl aliquot of medium before immunoprecipitation with β-endorphin antiserum. ●, β-Endorphin antiserum (RB100), cpm/slice; X, 5 μg of β-endorphin plus β-endorphin antiserum (RB100), cpm/slice.

immunoprecipitate. However, 90% of the 11.7k endorphin and 3.5k endorphin initially precipitable with the β-endorphin antiserum still appeared in the final immunoprecipitate. The same results were obtained when the two antisera were used in the opposite order (Fig. 2 *lower*). An initial immunoprecipitation with β-endorphin antiserum completely eliminated the 31k molecule from a subsequent ACTH α(1-24) immunoprecipitate; however, 85% of the 23k, 13k, and <4.5k ACTH initially precipitable with the ACTH α(1-24) antiserum still appeared in the final immunoprecipitate. Thus, the ACTH α(1-24) antiserum and the β-endorphin antiserum precipitated the same 31k molecule.

Our peptide studies indicated that 31k ACTH could contain a βLPH-like peptide that is cleaved from the COOH terminus of 31k ACTH during its conversion to 23k ACTH.‡ The fact that the 31k molecule is the only protein precipitated by both ACTH and endorphin antisera is consistent with this observa-

‡ B. A. Eipper and R. E. Mains, unpublished data.

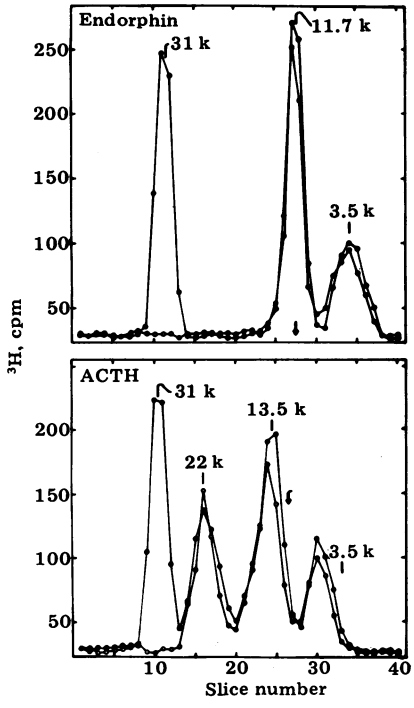

FIG. 2. Sequential immunoprecipitation of AtT-20 culture medium with antisera to ACTH and endorphin. The [^{3}H]phenylalanine-labeled culture medium described in Fig. 1 *upper* was used for the preparation of sequential immunoprecipitates. (*Upper*) Endorphin. Analysis of a direct immunoprecipitate from 10 μl of culture medium with β-endorphin antiserum (Fig. 1 *upper*) redrawn for comparison. An identical 10-μl aliquot of culture medium was subjected to an initial immunoprecipitation with purified ACTH α(1-24) antiserum; the supernatant from this initial immunoprecipitation was then immunoprecipitated with β-endorphin antiserum and this final immunoprecipitate was analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis. ●, β-Endorphin antiserum (RB100), cpm/slice; ○, sequential immunoprecipitation—β-endorphin (RB100) immunoprecipitation of supernatant from purified ACTH α(1-24) antiserum (Bertha) immunoprecipitation, cpm/slice. (*Lower*) ACTH. Analysis of a direct immunoprecipitate from 10 μl of culture medium with purified ACTH α(1-24) antiserum (Fig. 1 *upper*) redrawn for comparison. An identical 10-μl aliquot of culture medium was immunoprecipitated with β-endorphin antiserum; the supernatant from this initial immunoprecipitation was then mixed with nonimmune rabbit immunoglobulin and immunoprecipitated with purified ACTH α(1-24) antiserum. ●, Purified ACTH α(1-24) antiserum (Bertha), cpm/slice; ○, sequential immunoprecipitation—purified ACTH α(1-24) antiserum (Bertha) immunoprecipitation of supernatant from β-endorphin (RB100) immunoprecipitate, cpm/slice.

tion. The peptide segment cleaved from 31k ACTH to generate 23k ACTH could be approximately the size of βLPH (a 91-amino acid polypeptide); gel filtration in 6 M Gdn·HCl indicates that 31k ACTH contains about 260 amino acid residues whereas 23k ACTH contains about 160 amino acid residues (19). The structure of mouse βLPH has not been determined, but ovine, bovine, porcine, and human βLPH all contain a single tryptophan residue at position 52 (Fig. 3); β-endorphin does not contain a tryptophan residue. The 3.5k endorphin is similar to β-endorphin in that it contains phenylalanine but not tryptophan.

Separation of Forms of ACTH and Endorphin. In order to carry out any further analysis of the different forms of ACTH and endorphin, immunoprecipitates were solubilized and the forms were separated by gel filtration.‡ The purity of the separated forms as assessed by NaDodSO$_4$/polyacrylamide gel electrophoresis or by gel filtration in Gdn·HCl was greater than 95%. When analyzed in a phosphate-buffered NaDodSO$_4$/gel

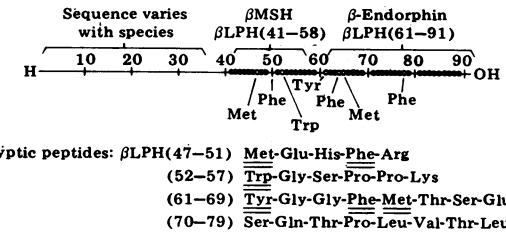

FIG. 3. Structure of βLPH and endorphins. The sequence data of Li and Chung (1) for human, ovine, and porcine βLPH are summarized schematically. The sequences of the tryptic peptides containing tryptophan, phenylalanine, and methionine are indicated. Methionine-enkephalin is identical to βLPH(61-65) (4); α-endorphin is βLPH(61-76) (6); γ-endorphin is βLPH(61-77) (7); β-endorphin is βLPH(61-91) (5).

electrophoresis system (22), 11.7k endorphin had a mobility similar to that of lima bean trypsin inhibitor (molecular weight, 9400); when analyzed by gel filtration on agarose A0.5m in 6 M Gdn·HCl (19), this same material had an apparent molecular weight of 8500.‡ The 3.5k endorphin was analyzed by gel filtration on a column of Sephadex G-50 in 6 M Gdn·HCl (19) and eluted at approximately the same position as synthetic ^{125}I-labeled β-endorphin.

Peptide Analysis. If the β-endorphin antiserum and the ACTH α(1-24) antiserum precipitate the same 31k molecule, the tryptic peptides of 31k ACTH should be identical to the tryptic peptides of 31k endorphin. The tryptic peptides of

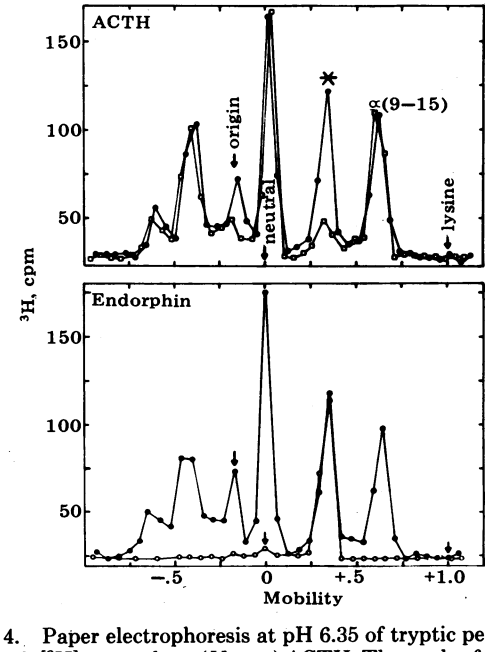

FIG. 4. Paper electrophoresis at pH 6.35 of tryptic peptides labeled with [^{3}H]tryptophan. (*Upper*) ACTH. The pools of [^{3}H]tryptophan-labeled 31k, 23k, and 13k ACTH prepared from immunoprecipitates of culture medium were greater than 90% pure. The patterns for 31k and 23k ACTH are shown. *, Position of the single [^{3}H]tryptophan-labeled tryptic peptide present in 31k ACTH but absent from 23k ACTH. The peptide labeled α(9-15) has the same size and charge properties as ACTH α(9-15) [the single major tryptophan-containing tryptic peptide in ACTH α_p(1-39)]. ●, 31k ACTH, cpm/slice; □, 23k ACTH, cpm/slice. (*Lower*) Endorphins. Pools of [^{3}H]tryptophan-labeled 31k and 11.7k endorphins were prepared from α-endorphin immunoprecipitates of culture medium. Tryptic peptides were prepared and analyzed as above; data for 31k and 11.7k endorphins were normalized by assuming that there are five tryptophan residues in 31k endorphin and one tryptophan residue in 11.7k endorphin.‡ ●, 31k endorphin, cpm/slice; ○, 11.7k endorphin, cpm/slice.

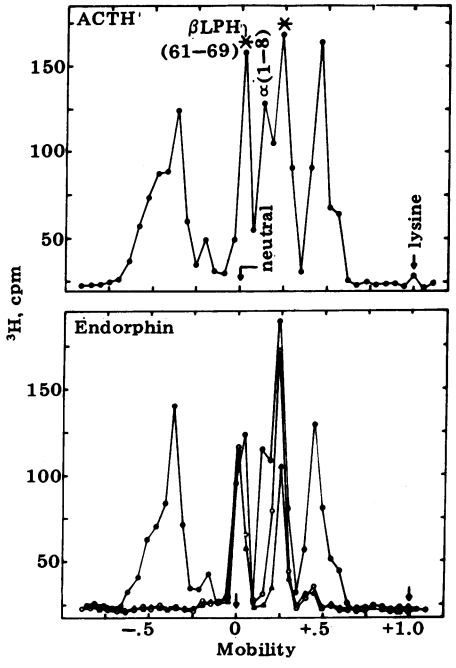

FIG. 5. Paper electrophoresis at pH 6.35 of tryptic peptides labeled with [^{3}H]phenylalanine. (*Upper*) 31k ACTH. Tryptic digests of [^{3}H]phenylalanine-labeled 31k, 23k, and 13k ACTH were analyzed by paper electrophoresis at pH 6.35; only the pattern obtained for 31k ACTH is shown. *, Position of peptides present in 31k ACTH but absent from 23k ACTH.‡ Tryptic digests of ACTH α_p(1-39) have two major phenylalanine-containing peptides [α(1-8) and α_p(22-39)]; the tryptic peptide labeled α(1-8) has the size and charge properties of ACTH α(1-8). ●, 31k ACTH, cpm/slice. (*Lower*) Endorphins. Tryptic digests of [^{3}H]phenylalanine-labeled 31k, 11.7k, and 3.5k endorphin (purified from α-endorphin immunoprecipitates) were prepared and analyzed as above. The data have been normalized by assuming that there are nine phenylalanine residues in the 31k endorphin, three in 11.7k endorphin, and two in 3.5k endorphin.‡ The two peaks of ^{3}H-labeled peptides occur in a ratio of 1.1:1.9 in 11.7k endorphin and 1.0:0.9 in 3.5k endorphin. βLPH(61-69) is the βLPH(or endorphin) tryptic peptide containing the methionine-enkephalin sequence; this peptide is neutral at pH 6.35. When the [^{3}H]phenylalanine-labeled tryptic digests of 11.7k and 3.5k endorphin are separated by electrophoresis at pH 3.5, each digest contains a positively charged peptide that has the same electrophoretic mobility as synthetic βLPH(61-69) (not shown). ●, 31k endorphin, cpm/slice; ○, 11.7k endorphin, cpm/slice; ▲, 3.5k endorphin, cpm/slice.

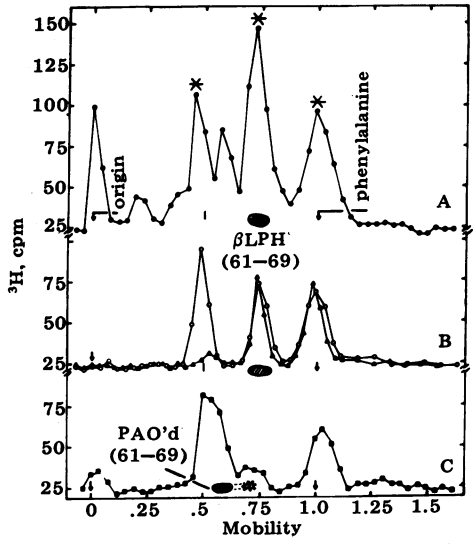

FIG. 6. Tryptic peptides prepared from pools of [^{3}H]phenylalanine-labeled endorphin (Fig. 5 *lower*) were analyzed by paper chromatography. (*A*) [^{3}H]Phenylalanine-labeled 31k endorphin tryptic peptides were chromatographed with 20 μg of synthetic βLPH(61-69) and 10 μg of phenylalanine; phenylalanine and βLPH(61-69) were visualized with ninhydrin. An identical profile was obtained with [^{3}H]phenylalanine-labeled 31k ACTH tryptic digests.‡ *, as in Fig. 5 *upper*. ●, 31k endorphin, cpm/slice. (*B*) Tryptic peptides of [^{3}H]phenylalanine-labeled 11.7k and 3.5k endorphin analyzed as described above; the data were normalized as described in Fig. 5 *lower*. The three tryptic peptides from 11.7k endorphin occurred in a ratio of 1.06:0.86:1.08; for 3.5k endorphin the ratio was 0.17:0.83:1.00. ○, 11.7k endorphin, cpm/slice; ▲, 3.5 k endorphin, cpm/slice. (*C*) Performic acid oxidation. Synthetic βLPH(61-69) (20 μg) was added to an aliquot of 11.7k endorphin tryptic peptides and the sample was oxidized with ice-cold performic acid for 4 hr (23). Oxidation of βLPH(61-69) converts the methionine residue at position 65 to methionine sulfone and decreases the mobility of the peptide in this chromatography system. The synthetic βLPH(61-69) oxidized along with the endorphin tryptic peptides was visualized with ninhydrin. The mobility of the [^{3}H]phenylalanine-labeled tryptic peptide that comigrated with βLPH(61-69) was altered in the same manner as the mobility of the synthetic peptide. ■, Oxidized tryptic peptides of 11.7k endorphin, cpm/slice.

[^{3}H]tryptophan- or [^{3}H]phenylalanine-labeled 31k ACTH and 31k endorphin were identical when analyzed by paper electrophoresis at pH 6.35 (Figs. 4 and 5). The identity of 31k ACTH and 31k endorphin was confirmed by analysis of [^{3}H]-phenylalanine-labeled tryptic peptides by paper chromatography (Fig. 6*A*) and by electrophoretic and chromatographic analysis of [^{3}H]phenylalanine-labeled chymotryptic peptides (not shown).

Analysis of tryptic digests of 31k ACTH labeled with tryptophan, tyrosine, lysine, arginine, methionine, and phenylalanine has shown that the 31k molecule contains all of the tryptic peptides of ACTH α(1-39).‡ If 11.7k endorphin represents most of the material cleaved from 31k ACTH when it is converted to 23k ACTH, peptides that appear in tryptic digests of 31k ACTH but not in tryptic digests of 23k ACTH should appear in 11.7k endorphin. In addition, if 11.7k endorphin resembles βLPH and 3.5k endorphin resembles β-endorphin, their tryptic peptides should be similar to the tryptic peptides of βLPH and β-endorphin.

Tryptic digests of [^{3}H]tryptophan-labeled 11.7k endorphin contained a single labeled tryptic peptide (Fig. 4 *lower*); this tryptic peptide from 11.7k endorphin had the same electrophoretic mobility as the [^{3}H]tryptophan-labeled tryptic peptide present in 31k ACTH but absent from 23k ACTH (analyzed at pH 6.35 and at pH 3.5). The size (determined by gel filtration in Gdn·HCl) and charge properties of the [^{3}H]tryptophan-labeled tryptic peptide were similar to those expected of the single tryptophan-containing tryptic peptide in βLPH (residues 52–57) as predicted by the method of Offord (24).

Tryptic digests of [^{3}H]phenylalanine-labeled 11.7k endorphin contained equal amounts of three labeled peptides (Fig. 5 *lower* and 6*B*). These three peptides were a subset of the [^{3}H]phenylalanine-labeled peptides present in the 31k molecule; tryptic digests of 23k ACTH do not contain this set of peptides.‡ Tryptic digests of [^{3}H]phenylalanine-labeled 3.5k endorphin contained two of the three peptides present in 11.7k endorphin. A βLPH-like molecule should contain three phenylalanine-containing tryptic peptides whereas a β-endorphin-like molecule should contain two (Fig. 3). One phenylalanine-containing tryptic peptide that should be present in both a βLPH-like molecule and a β-endorphin-like molecule is the tryptic nonapeptide with the sequence of methionine-enkephalin at its NH_2 terminus [βLPH(61-69)]. When synthetic βLPH(61-69) was included in the chromatographic separation of tryptic digests of [^{3}H]phenylalanine-labeled endorphins, it comigrated with one of the ^{3}H-labeled peptides (Fig. 6 *A* and

B); this [^{3}H]phenylalanine-labeled tryptic peptide migrated with synthetic βLPH(61-69) during electrophoresis at pH 3.5 and 6.35, and gel filtration in 6 M Gdn·HCl indicated that it had 9 or 10 residues. Performic acid oxidation altered the chromatographic mobility of synthetic βLPH(61-69) and the corresponding [^{3}H]phenylalanine-labeled tryptic peptide from 11.7k endorphin in the same manner (Fig. 6 *C*). Comparison of the tryptic peptides of [^{3}H]methionine-labeled 31k ACTH and 23k ACTH also indicates that this peptide contains methionine.‡ A [^{3}H]tyrosine-labeled tryptic peptide that migrated with βLPH(61-69) in two chromatographic and two electrophoretic systems could be isolated from tryptic digests of [^{3}H]tyrosine-labeled 31k ACTH in good yield; performic acid oxidation of this [^{3}H]tyrosine-labeled peptide along with a sample of synthetic βLPH(61-69) altered the mobility of both peptides in a similar manner. The other [^{3}H]phenylalanine-containing tryptic peptide common to 11.7k and 3.5k endorphins should resemble βLPH(70-79); the size and charge properties of this peptide were consistent with this similarity. The [^{3}H]phenylalanine-containing tryptic peptide present in 11.7k endorphin but absent from 3.5k endorphin should resemble βLPH(47-51) (Fig. 3). The size and charge properties of this [^{3}H]phenylalanine-containing tryptic peptide were similar to those predicted for βLPH(47-51), but performic acid oxidation (Fig. 6*C*) and labeling experiments with [^{3}H]methionine indicated that it contained no methionine.

DISCUSSION

Sequential immunoprecipitation and analysis of peptides indicate that 31k ACTH is identical to 31k endorphin; 31k ACTH is the biosynthetic precursor of the smaller forms of ACTH and has the properties expected of a pro-corticotropin (18). The 31k molecule is the only molecule that contains antigenic determinants for both ACTH and endorphins (Figs. 1 and 2); 31k ACTH/endorphin can be converted to 23k ACTH plus 11.7k endorphin (Figs. 4–6). The 11.7k endorphin is similar in structure to βLPH; 3.5k endorphin is similar to β-endorphin. If 31k ACTH/endorphin is used to generate both endorphin- and ACTH-related peptides and if these peptides are stable in the culture medium, the medium should contain equimolar amounts of endorphin (11.7k and 3.5k) and ACTH (23k, 13k, and <4.5k). Peptide analyses make it possible to determine the number of phenylalanine or tryptophan residues in each form of ACTH and endorphin (Figs. 4–6). From this information it is possible to calculate that the culture medium contains 80–90% as many moles of endorphin-related peptides as moles of ACTH-related peptides (Fig. 1). Although there is evidence that, under many circumstances, approximately equimolar amounts of ACTH and LPH appear in plasma (8–11), it is not clear that the 31k ACTH/endorphin molecule is always used to generate both ACTH and endorphins; selective release of ACTH or endorphins could be achieved by proteolysis of the appropriate molecules.

The peptide data presented here indicate that mouse pituitary tumor cell 11.7k endorphin is similar but not identical to human, bovine, ovine, and porcine βLPH. The 11.7k endorphin does not contain a methionine residue equivalent to the methionine at position 47 of βLPH. βLPH and βMSH have not been purified from mouse or rat tissue. Analysis of extracts of rat and mouse pituitary and AtT-20 cells with βMSH immunoassays indicate that mouse and rat βMSH and βLPH are not identical to the human, porcine, bovine, or ovine molecules (3, 25–27).

The fact that ACTH and endorphins can be derived from a single common precursor molecule suggests new ways to interpret the function of pituitary endorphins.

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