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Corticotropin, lipotropin, and β-endorphin production by a human nonpituitary tumor in culture: Evidence for a common precursor

(pulmonary small cell carcinoma/radioimmunoassay/gel filtration/guanidine hydrochloride/affinity chromatography)

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ABSTRACT **A continuous line (DMS-79) of human pulmonary small cell carcinoma cells was shown to secrete immunoreactive adrenocorticotropin (ACTH), lipotropin, and β-endorphin concomitantly into the culture medium. Gel filtration of the culture medium demonstrated at least five components: high molecular weight material(s) that had ACTH, lipotropin, and β-endorphin immunoreactivities and materials similar to ACTH, β-lipotropin, γ-lipotropin, and β-endorphin in their immunoreactivities and apparent molecular weights. The same components were observed when gel filtration was carried out in 6 M guanidine·HCl, and the high molecular weight material(s) appeared to consist of more than one component, with molecular weights in the range of 15,000–40,000. Immune affinity chromatography of the high molecular weight component(s) from gel filtration with a specific anti-(1–24)ACTH serum demonstrated that the ACTH, lipotropin, and β-endorphin immunoreactivities were possessed by the same molecule(s), suggesting that ACTH, lipotropins, and β-endorphin were derived from a common, high molecular weight precursor.**

It has been reported that mouse pituitary tumor cell line AtT-20/D-16v secretes an adrenocorticotropin (ACTH)-like component, a β-lipotropin (βLPH)-like component, a β-endorphin (βEnd)-like component (1–3) and a high molecular weight (M_r ~31,000) glycopeptide, designated M_r 31,000 ACTH (4). This M_r 31,000 ACTH is thought to be a common precursor for the ACTH, βLPH, and βEnd produced by this cell (2, 3, 5).

That such a common precursor also exists in man remains to be demonstrated, although there is already some evidence for the existence of a similar biosynthetic pathway (6–15).

We have studied a continuous line of human pulmonary small cell carcinoma cells in culture (16, 17), using gel filtration chromatography and radioimmunoassays (RIAs) for ACTH, hLPHs, hγLPH, and hβEnd to demonstrate that the culture medium contained components similar to ACTH, hβLPH, hγLPH and hβEnd and a large component(s) whose apparent molecular weight and immunological properties suggest that it represents a common precursor for all of these hormones.

MATERIALS AND METHODS

Human Cell Culture. The DMS-79 cell line originated from pleural fluid obtained from a patient with small cell anaplastic carcinoma of the lung (16; O. S. Pettengill, G. D. Sorenson, and L. H. Maurer, unpublished results). Cells were grown in stationary suspension in RPMI 1640 medium (GIBCO) with 20% fetal calf serum [Rehatuin (Reheis Chemical Co.) heat inactivated at 56° for 30 min] at 37° in 5% CO_2/95% air. Cultures were subdivided at approximately weekly intervals by gentle mechanical dissociation of cells.

For the time-course experiment, 4.7×10^4 cells per ml of fresh RPMI 1640 medium with 20% fetal calf serum were incubated in stationary suspension cultures for 4 days. The cells were agitated, and portions of the cell suspension were removed every 24 hr. Cells were dispersed and counted in a hemocytometer. Hormone RIAs were performed on unextracted medium after the cells had been removed by centrifugation.

Two different pools of medium in which the cells were grown for 3–4 days were used for immunologic and chromatographic studies. The cell density at the completion of these incubations was approximately 1×10^6 cells per ml. After being harvested, the media were diluted with an equal volume of acetic acid, heated for 10 min at 90°, diluted with 4 vol of deionized glass-distilled water, lyophilized, and stored at −70°.

Radioimmunoassays. (*i*) *ACTH RIA.* This RIA, using antiserum S_1B_2 directed toward the 11–24 sequence of ACTH not shared with other known hormones, was performed as described (18) with the following modifications: (*a*) a stock solution of buffer A (0.063 M Na_2HPO_4, pH 7.4/0.0127 M disodium EDTA/and 100 mg of Merthiolate per liter was prepared; buffer B [used in place of standard diluent (18)] was prepared prior to each assay by adding ACTH-free human plasma (10 ml/liter), lysozyme (1 g/liter) and Trasylol (300 kallikrein inactivator units/ml) to buffer A; (*b*) labeled hACTH was purified by Sephadex G-50 fine (Pharmacia) gel filtration; and (*c*) separation of antibody-bound hormone from free hormone was achieved by precipitation with a second antibody.

(*ii*) *Human β-Endorphin RIA.* Anti-ovine βEnd serum RB100-11/15 (19) was used for the hβEnd RIA. Synthetic hβEnd (Bachem, Inc., Torrance, CA) was used for iodination and as standard; that used for iodination was stored at −70° in 5 mM HCl and that for standard, at −70° in buffer C [0.02 M Na_2HPO_4/KH_2PO_4, pH 7.4/0.1 M NaCl containing 1 g of USP gelatin, 100 mg of bovine serum albumin (Miles), and 100 mg of Merthiolate per liter]; further dilutions were made in buffer D (buffer C containing 1 ml of Triton X-100 per liter). ^{125}I-Labeled hβEnd was prepared by the method described for hACTH (18); a specific activity of 650 μCi/μg was obtained. It was purified by Sephadex G-50 fine gel filtration prior to each assay. Mixtures were incubated for 3 days at 4°; separation of antibody-bound hormone from free hormone was achieved by precipitation with a second antibody.

(*iii*) *Human LPH RIA.* The hLPH RIA procedure, using antiserum R-3 (20) and synthetic (37–58)hLPH ["human β-melanotropin (β-MSH)," Ciba–Geigy] as tracer and standard

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Abbreviations: hACTH, human ACTH; hβEnd, human β-endorphin; hβLPH, human β-lipotropin; MSH, melanotropin; hγLPH, human γ-lipotropin; Gdn·HCl, guanidine·HCl; HMW, high molecular weight; IR, immunoreactive; RIA, radioimmunoassay; DNP-alanine, 2,4-dinitrophenyl-alanine. Fragments of ACTH and other peptides are indicated by a prefix listing their inclusive amino acid residues in parentheses [e.g., (1–24)ACTH is the NH_2-terminal 24-amino acid fragment of ACTH].

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(20), was modified in exactly the manner described for ACTH RIA except that labeled tracer was purified by Sephadex G-25 fine gel filtration.

(*iv*) *Human γLPH RIA.* The hγLPH RIA procedure was identical to that described for hLPH RIA. Antiserum R1547 was obtained by injecting a rabbit with synthetic (37–58)hLPH coupled to albumin by the glutaraldehyde reaction (18). Highly purified hγLPH (P. J. Lowry) was used as the standard.

Gel Filtration Chromatography. A 1.5 × 90-cm column was packed with Sephadex G-50 fine gel, which was equilibrated and developed in buffer B at 4°. The flow rate was 22 ml/hr; 2.2-ml fractions were collected and stored at −70° until assay. The following reagents were used to calibrate the column: bovine serum albumin; highly purified hβLPH (C. H. Li) and hγLPH; synthetic hACTH (Ciba-Geigy), (37–58)hLPH, and hβEnd; and NaCl.

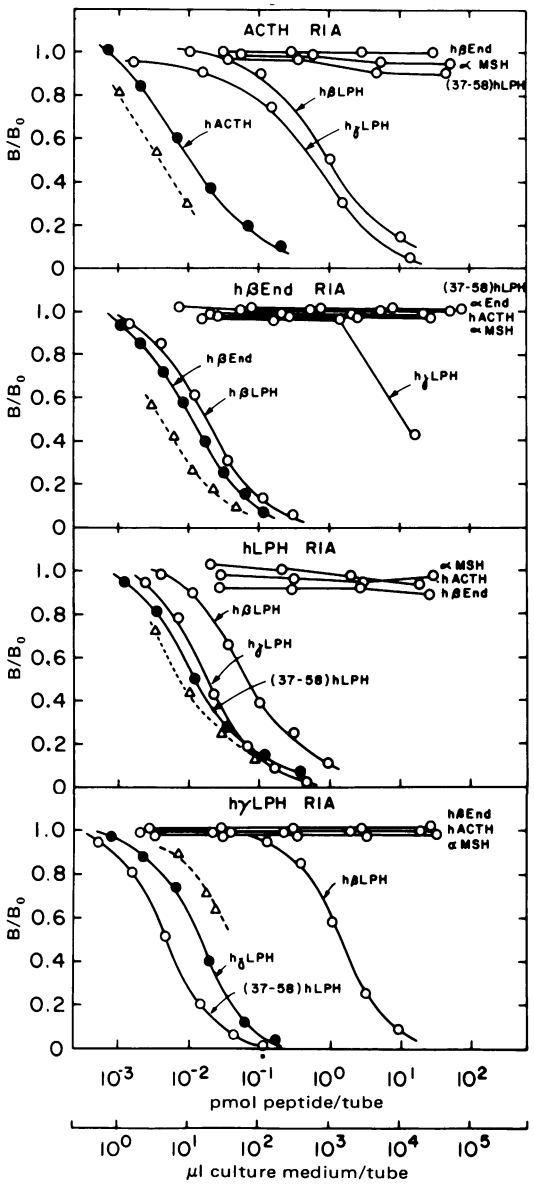

FIG. 1. ACTH, hβEnd, hLPH, and hγLPH radioimmunoassay specificity. The competitive binding curves generated by the synthetic hACTH, hβEnd, (37–58)hLPH, and highly purified hγLPH standards (●—●) and the relative immunoreactivities of these peptides, synthetic αMSH and αEnd, and highly purified hβLPH (○—○) are shown. Data are plotted as B/B_0 vs. log pmol of peptide added per tube. The competitive binding curves generated by serial dilutions of the tumor culture medium in each assay are also shown (△ - - - △). The concentrations in the medium were 2.4 pmol of IR-ACTH, 3.2 pmol of IR-hβEnd, 1.5 pmol of IR-hLPH, and 0.38 pmol of IR-hγLPH, all per ml; the concentrations of these IR peptides in RPMI 1640 medium containing 20% fetal calf serum prior to incubation were 0.020, 0.072, 0.022, and 0.072 pmol per ml, respectively.

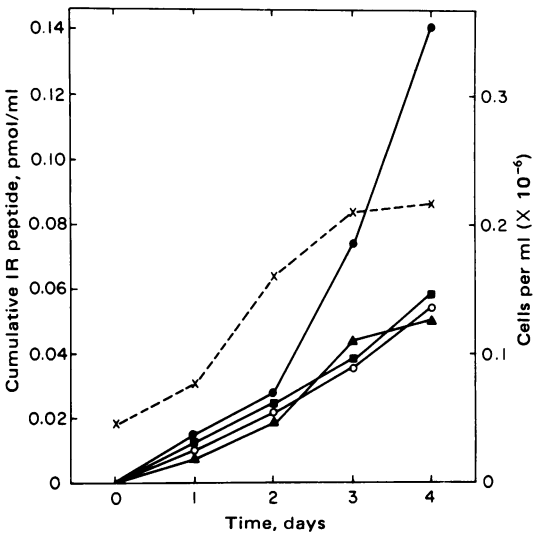

FIG. 2. Time-course of DMS-79 cell growth and IR peptide accumulation in medium. DMS-79 cells were dispersed in fresh medium (4.7×10^4 cells per ml) and incubated in stationary suspension culture. Cell number (×) and cumulative concentrations of IR-ACTH (●), IR-hLPH (■), IR-hγLPH (○), and IR-hβEnd (▲) are indicated. The concentration of each IR component in control medium containing no cells has been subtracted from each data point.

Gel Filtration Chromatography under Denaturing Conditions. A 1.5 × 90-cm column was packed with Sepharose CL-6B (Pharmacia) equilibrated and developed at 4° with two buffers: (*i*) 6 M guanidine·HCl (Gdn·HCl; ultrapure, Schwarz/Mann)/0.1 M Na_2HPO_4, containing 200 mg of albumin per liter, pH adjusted to 7.0 with 1 M NaOH; and (*ii*) 6 M Gdn·HCl/0.001 M dithiothreitol (Calbiochem)/0.5 M Tris·HCl, containing 200 mg of albumin per liter, pH 8.1 (21). One milliliter of sample or a single standard was applied and eluted by ascending flow; fraction volume was determined by weight. The column was calibrated with: blue dextran 2000 (Pharmacia); reduced and carboxymethylated ovalbumin (Sigma); reduced and carboxymethylated myoglobin (Sigma); cytochrome *c* (Sigma); highly purified hβLPH and hγLPH; synthetic hACTH, ^{125}I-hβEnd, and (37–58)hLPH; and 2,4-dinitrophenyl-N-alanine (DNP-alanine, Sigma). Each sample or standard was incubated either in the 6 M Gdn·HCl buffer for 18 hr at 4° or in 6 M Gdn·HCl/0.5 M Tris·HCl, pH 8.1/1mM EDTA/0.01 M dithiothreitol for 4 hr at 50° prior to application; protein concentration was 3%. Each sample contained blue dextran 2000 and DNP-alanine as internal markers.

Affinity Chromatography. (*i*) *Preparation of Anti-ACTH Serum.* Rabbit R-1549 was injected with (1–24)ACTH (Cortrosyn, Organon) conjugated to albumin using glutaraldehyde (18).

(*ii*) *Coupling (1–24)ACTH to Sepharose.* One gram of CNBr-Sepharose 4B (Pharmacia) was prewashed with 200 ml of 1 mM HCl and reacted with 7.1 mg of (1–24)ACTH in 5 ml of 0.1 M $NaHCO_3$, pH 8.5/0.4 M NaCl for 2 hr at room temperature with end-over-end mixing (22). Excess reactive groups were blocked by adding 0.1 M Tris·HCl, pH 8.5/0.4 M NaCl and continuing the reaction an additional 2 hr. The gel was then washed with 0.1 M ammonium acetate, pH 4.0/1 M NaCl containing 100 mg of Merthiolate per liter and stored at 4°.

(*iii*) *Purification of Antiserum.* A column containing 150 μg of coupled (1–24)ACTH and enough Sepharose 4B gel to provide a bed volume of 0.5 ml was washed with 4 ml of 6 M Gdn·HCl (pH 1.5) and equilibrated with Buffer E (0.05 M

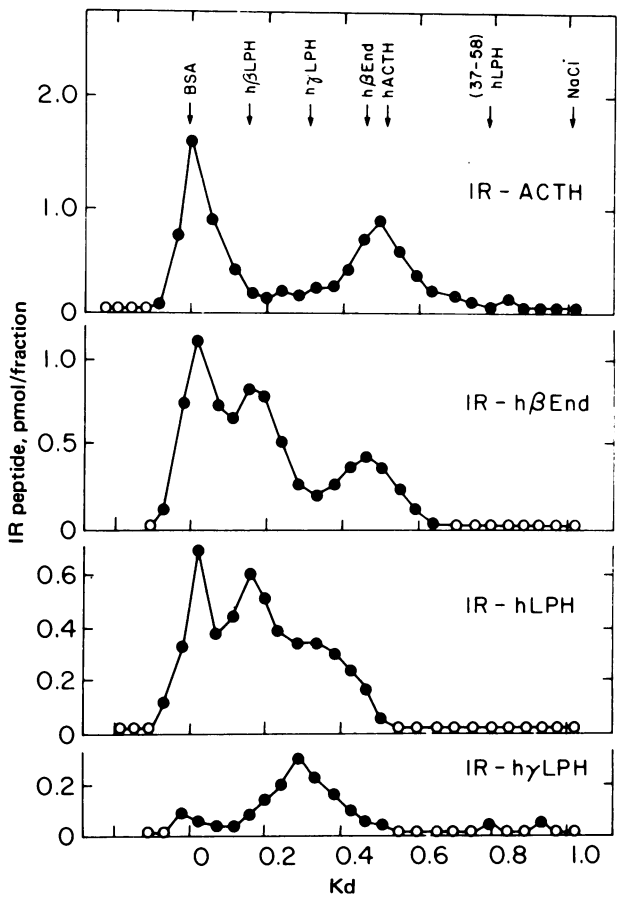

FIG. 3. Sephadex G-50 gel filtration of DMS-79 medium. The equivalent of 10 ml of medium was reconstituted from lyophilized powder in 4 ml of distilled water, aliquots were removed for hormone RIA, and the remaining 3.7 ml were applied to the 1.5 × 90 cm G-50 column. The 2.2-ml fractions collected were stored at −70° until assayed. Recoveries of IR-hACTH, IR-hLPH, IR-hγLPH, and IR-hβEnd were 82, 132, 89, and 76%, respectively. Kd = fractional elution volume. o, Nondetectable immunoreactivity.

Na_2HPO_4/0.05 M NaCl, pH adjusted to 7.4 with 1 M HCl). A solution of 5 ml of antiserum R-1549 and 5 ml of buffer E was applied, the column was washed with 3 ml of buffer E, and the purified antibody was eluted with 3 ml of 6 M Gdn·HCl, pH 1.5 (23). The antibody was dialyzed twice against buffer E and once against 0.1 M $NaHCO_3$, pH 8.5/0.4 M NaCl.

(*iv*) *Preparation of Anti-ACTH Column.* The purified antibody was reacted with 150 mg of CNBr-Sepharose 4B as described above for (1–24)ACTH. A column was prepared by mixing the Sepharose-bound antibody with enough Sepharose 4B to provide a bed volume of 0.5 ml. Immediately before each use, the column was washed with 4 ml of 6 M Gdn·HCl (pH 1.5) and 4 ml of buffer E and was equilibrated with 4 ml of buffer B. The sample was then applied, followed by 2 ml of buffer B, 1 ml of buffer E, 3 ml of buffer D, 1 ml of buffer E, and 3 ml of 1 M NaCl. Immunoadsorbed materials were eluted with 4 ml of 6 M Gdn·HCl, pH 1.5. The flow rate was 12 ml/hr.

(*v*) *Specific Elution with Excess (1–24)ACTH.* One-eighth of the above column was diluted with Sepharose 4B to provide a bed volume of 0.5 ml. Samples to which approximately 1 × 10^4 cpm of ^{125}I-labeled hACTH had been added were applied, and the column was washed as described above. Immunoadsorbed materials were eluted with 2 ml of Buffer D containing 10 μg of (1–24)ACTH per ml, followed by 2 ml of buffer D at a flow rate of 1 ml/hr. The reduced capacity and flow rate were necessary for the (1–24)ACTH to cause maximal displacement. The adsorbed materials were eluted with 4 ml of 6 M Gdn·HCl (pH 1.5) at a flow of 12 ml/hr.

Preparation of Samples for Radioimmunoassay. Fractions from the Sephadex G-50 column were assayed after dilution in the appropriate RIA buffers. Fractions containing Gdn·HCl or NaCl were desalted prior to assay as described (24).

RESULTS

Radioimmunoassay Specificity. The specificity of each RIA is shown in Fig. 1. The ACTH RIA was specific for the (11–24)ACTH sequence. The minor crossreactivities of hβLPH and hγLPH probably resulted from slight contamination of these purified pituitary preparations with ACTH.

The hLPH RIA reacted with both hβLPH and hγLPH. However, their different potencies (20% and 68% on a molar basis, respectively) with respect to standard (37–58)hLPH did not permit an exact estimate of concentrations in medium or column fractions. Therefore, the hLPH RIA results have been expressed as pmoles of (37–58)hLPH standard and represent a variable underestimate of actual molar concentration.

The hβEnd RIA is directed toward the COOH-terminal portion (19) of a polypeptide that is identical to the 31-amino acid COOH-terminal sequence of βLPH. Therefore, hβLPH reacted on an almost equimolar basis with hβEnd, and hβLPH and hβEnd must be physically separated in order to determine with which of them this antibody is reacting.

In the hγLPH RIA, hβLPH was less than 1% as immunoreactive (IR) as hγLPH, presumably because the antibodies reacted with the free COOH terminus of hγLPH (20).

Serial dilutions of pooled medium in which DMS-79 cells had been cultured generated competitive binding curves parallel to those of the standard peptides in all four RIAs.

Time-Course of Cell Growth and Peptide Accumulation. DMS-79 cell number increased steadily for 3 days and then appeared to plateau (Fig. 2). Cumulative concentrations of IR peptides increased in parallel, except for a late disproportionate increase in IR-ACTH.

Sephadex G-50 Gel Filtration Chromatography. Gel fractionation of culture medium on Sephadex G-50 revealed several different components that were responsible for the observed immunoreactivities of the medium in the four RIAs (Fig. 3). There was a high molecular weight (HMW) component(s) that had ACTH, hβEnd, hLPH, and hγLPH immunoreactivities; a component that coeluted with hβLPH and had both hβEnd and hLPH immunoreactivities and appeared, therefore, to be a substance similar to hβLPH itself; a shoulder of IR-hLPH that had hγLPH immunoreactivity and coeluted with hγLPH and thus appeared to be a substance similar to hγLPH; a peak of IR-hβEnd that coeluted with synthetic hβEnd; and a peak of IR-ACTH that coeluted with synthetic hACTH. No IR-hLPH component was observed that eluted in the position of synthetic (37–58)hLPH ("human βMSH").

Sepharose CL-6B/Gdn·HCl Gel Filtration Chromatography. The same immunoreactive components were observed under denaturing conditions (Fig. 4*a*). The HMW material(s) eluted as a broad peak (M_r 15,000 to 40,000, Kd = 0.40–0.55). Substances similar to hβLPH, hACTH, and hβEnd were again observed, and probably an hγLPH-like substance. In the presence of dithiothreitol, the HMW substance(s) in a second pool of DMS-79 medium eluted in the same volume (Kd = 0.40–0.55; Fig. 4*b*) and retained ACTH, hLPH, and hβEnd immunoreactivities. Substances similar to hβLPH and probably hγLPH were also found, but definite peaks of ACTH- and hβEnd-like substances were lacking in this medium.

Affinity Chromatography. Most of the ^{125}I-hACTH was adsorbed to the anti-ACTH affinity column and eluted with acidified 6 M Gdn·HCl (Fig. 5*a*). In contrast, ^{125}I-hβEnd and a mixture of ^{125}I-hβLPH and ^{125}I-hγLPH were almost totally excluded, demonstrating the specificity of the column.

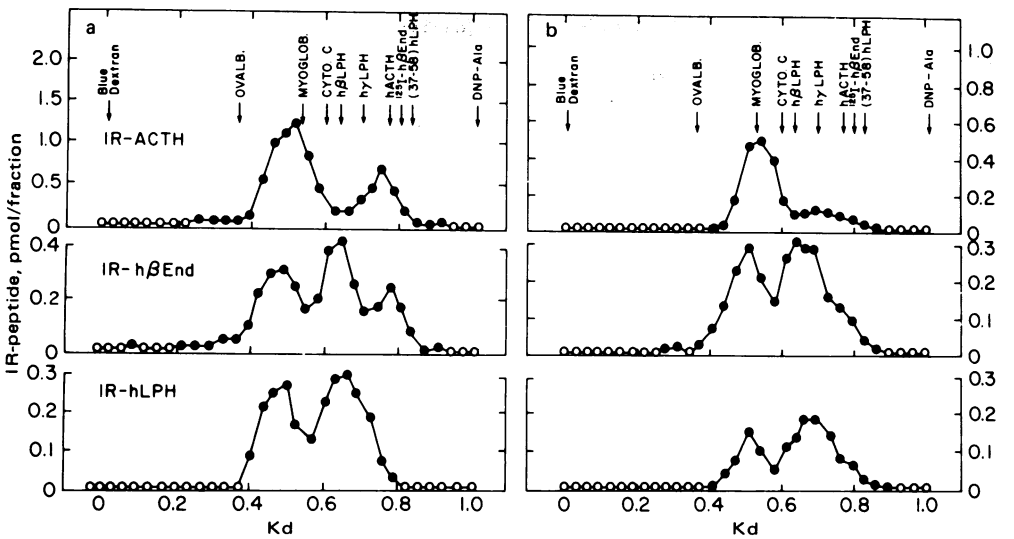

FIG. 4. Sepharose CL-6B chromatography of DMS-79 culture medium in 6 M Gdn·HCl. (*a*) Lyophilized pooled DMS-79 culture medium (81.6 mg, equivalent to 36 mg of protein) was dissolved in 1.2 ml of 6 M Gdn·HCl in 0.1 M phosphate buffer, pH 7.0, containing 2.0 mg of blue dextran 2000 and 0.250 mg of DNP-alanine, and was incubated at 4° for 18 hr. Four 50-μl aliquots were removed to determine their IR hormone content, and the remainder (1.0 ml) was applied to the column. Alternate fractions and the 50-μl aliquots were desalted and assayed in the three RIAs. Recoveries of IR-ACTH, IR-hLPH, and IR-hβEnd were 87, 83, and 98%, respectively. (○), Nondetectable immunoreactivity. (*b*) Lyophilized medium from a second pool (75 mg, equivalent to 36 mg of protein) was dissolved in 1.2 ml of 6 M Gdn·HCl in 0.5 M Tris·HCl, pH 8.1/1 mM EDTA/0.01 M dithiothreitol and was incubated at 50° for 4 hr. Blue dextran 2000 and DNP-alanine were added to the sample after the incubation, and the sample was processed in the same manner as above, except that the column was eluted with the 6 M Gdn·HCl/1 mM dithiothreitol buffer (see *Materials and Methods*). Recoveries of IR-ACTH, IR-hLPH, and IR-hβEnd were 98, 83, and 99%, respectively. (○), Nondetectable immunoreactivity.

When pooled fractions from the HMW region (Kd = 0.40–0.53) of the Sepharose CL-6B/Gdn·HCl column (Fig. 4*a*) were applied to the affinity column, less than 19% of the IR-ACTH, IR-hLPH, or IR-hβEnd were excluded, and similar percentages of each (96, 85, and 73%, respectively) were eluted with acidified 6 M Gdn·HCl (Fig. 5*b*).

When pooled HMW fractions (Kd = 0.40–0.53) from the Sepharose Cl-6B/Gdn·HCl-dithiothreitol column (Fig. 4*b*) were applied to the low-capacity affinity column, the IR-hLPH and IR-hβEnd were still largely adsorbed (Fig. 5*c*). Excess (1–24)ACTH specifically displaced essentially all of the adsorbed IR-hLPH and IR-hβEnd, as well as the ^{125}I-hACTH tracer.

DISCUSSION

A continuous line of human pulmonary small cell carcinoma cells, DMS-79, has been established in tissue culture (16, 17). Although this tumor was obtained from a patient who was not suspected of having the "ectopic ACTH syndrome" (25), the tumor cells in culture produced IR-ACTH (17). Thus, this cell line appeared to provide a potential model in which to examine the biosynthetic mechanism whereby ACTH, LPHs, and βEnd are produced in man. This potential appeared even greater when we found similar concentrations of IR-ACTH, IR-hLPH, IR-hγLPH, and IR-hβEnd in DMS-79 culture medium. Furthermore, the time-course of appearance of all four IR components in the medium was virtually identical for 2 days.

After comparing the Sephadex G-50 elution volumes of the IR components with those of standard hormones, it appeared there were at least five different IR components in the DMS-79 cell culture medium: substances similar to hACTH, hβEnd, hβLPH, hγLPH and HMW material(s) with ACTH, hLPH, hγLPH, and hβEnd immunoreactivities.

Because gel filtration in neutral buffer does not exclude aggregation, noncovalent binding to larger molecules, or configurational changes as causes for apparent increased molecular weight (26), gel filtration in buffer containing 6 M Gdn·HCl as a denaturing agent (27) was performed (Fig. 4*a*). Under these conditions, the same five components were again identified, with appropriate immunoreactivities and elution volumes. The HMW material(s) eluted in a broad peak in the 15,000–40,000-M_r range. The breadth of this peak suggested that it consisted of more than one IR component. In addition to 31,000-dalton ACTH, two intermediate-sized ACTH molecules have been characterized in AtT-20/D-16v cells and culture medium (5), and an intermediate-sized ACTH has also been identified in human tissue extracts (14).

In the presence of a reducing agent, the HMW component(s) eluted in the same fractions and retained their ACTH, hLPH and hβEnd immunoreactivities (Fig. 4*b*). The lack of distinct peaks of ACTH- and hβEnd-like materials under these conditions does not appear to be due to loss of these peptides under reducing conditions, because 63 and 100% of IR-ACTH and IR-hβEnd, respectively, were recovered from whole culture medium after incubation for 4 hr at 50° in 6 M Gdn·HCl/1 mM EDTA/0.01 M dithiothreitol, pH 8.1. It seems more likely that the second pool of culture medium did not contain these components, possibly because of selective degradation of these small peptides in the medium during longer incubation periods. These data do demonstrate that components possessing ACTH, hLPH, and hβEnd immunoreactivity coelute as HMW moieties under conditions that eliminate noncovalent binding; the apparent molecular weight range of the HMW IR components encompasses those observed in mouse pituitary tumor cells.

Finally, we wished to determine if the ACTH, hLPH, and hβEnd immunoreactivities in the HMW component(s) were all possessed by the same or by different molecules. Mains *et al.* used sequential immunoprecipitation to demonstrate that AtT-20/D-16v M_r 31,000 ACTH contained both ACTH and βEnd immunoreactivities (2); we used immune affinity chromatography for the same purpose. The affinity column contained ACTH antibodies that had themselves been purified by (1–24)ACTH affinity chromatography and that, in the presence of ACTH concentrations even higher than those measured by RIA in the culture medium, bound most of added ^{125}I-labeled hACTH and only negligible amounts of added labeled hβLPH, hγLPH, and hβEnd (Fig. 5*a*). In contrast, when pooled frac-

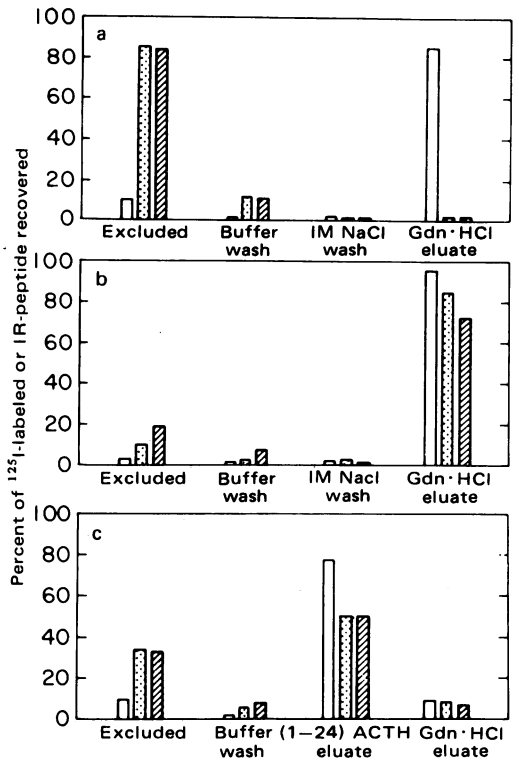

FIG. 5. Anti-(1-24)ACTH immune affinity chromatography. (*a*) Recovery of freshly prepared ^{125}I-hACTH (□), ^{125}I-hβEnd (▣), and a mixture of ^{125}I-hβLPH and -hγLPH (▨) applied to the anti-(1–24)ACTH affinity column in the presence of 1 μg of unlabeled hACTH. Total recovery of ^{125}I cpm was 91, 98, and 99%, respectively. Results are expressed as percent of total recovery cpm. (*b*) Even-numbered fractions (Kd = 0.40–0.53) from the Sepharose CL-6B/Gdn·HCl column (Fig. 4*a*) were desalted and pooled. After aliquots were removed for RIAs, the pool was applied to the anti-(1–24)ACTH affinity column as described in *Materials and Methods*. Four fractions were collected: the excluded fraction, the buffer wash, the 1 M NaCl wash, and the fraction eluted with 6 M Gdn·HCl, pH 1.5. The last two were desalted, and IR hormone content of the applied sample and each fraction was assayed simultaneously. Total recovery of IR-ACTH (□), IR-hβEnd (▣), and IR-hLPH (▨) was 80, 87, and 86%, respectively. Results are expressed as percent of total recovered IR peptide. (*c*) Even-numbered fractions (Kd = 0.40–0.53) from the Sepharose CL-6B/Gdn·HCl-dithiothreitol column (Fig. 4*b*) were processed as described above, except that freshly prepared ^{125}I-hACTH tracer was added to the sample before application. The adsorbed peptides were specifically eluted with 2 ml of buffer containing unlabeled (1–24)ACTH at 10 μg/ml. Recovery of ^{125}I-hACTH (□), IR-hβEnd (▣), and IR-hLPH (▨) was 98, 81, and 132%, respectively. Results are expressed as percent of recovered peptide.

tions from the HMW region of the Sepharose CL-6B/Gdn·HCl column eluate were applied to the ACTH affinity column, most of the ACTH, hLPH, and hβEnd immunoreactivity was bound to the column and eluted only with acidified 6 M Gdn·HCl (Fig. 5*b*) or an excess of (1–24)ACTH (Fig. 5*c*). It appears, therefore, that most, if not all, of the hLPH and hβEnd HMW component(s) are contained within a molecule(s) that also contains the ACTH antigenic sequence.

This work demonstrates concomitant secretion of ACTH, βLPH, γLPH, and βEnd by a human cell line in tissue culture. The data suggest that, as in the AtT-20/D-16v cell, all of these peptides are derived from a common precursor molecule.

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1. Eipper, B. A. & Mains, R. E. (1975) *Biochemistry* **14**, 3836–3844.
2. Mains, R. E., Eipper, B. A. & Ling, N. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3014–3018.
3. Roberts, J. L. & Herbert, E. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5300–5304.
4. Eipper, B. A., Mains, R. E. & Guenzi, D. (1976) *J. Biol. Chem.* **251**, 4121–4126.
5. Mains, R. E. & Eipper, B. A. (1976) *J. Biol. Chem.* **251**, 4115–4120.
6. Yalow, R. S. & Berson, S. A. (1971) *Biochem. Biophys. Res. Commun.* **44**, 439–445.
7. Yalow, R. S. & Berson, S. A. (1973) *J. Clin. Endocrinol. Metab.* **36**, 415–423.
8. Gewirtz, G. & Yalow, R. S. (1974) *J. Clin. Invest.* **53**, 1022–1032.
9. Orth, D. N. & Nicholson, W. E. (1977) *Ann. N. Y. Acad. Sci.* **297**, 27–48.
10. Lowry, P. J., Rees, L. H., Tomlin, S., Gillies, G. & Landon, J. (1976) *J. Clin. Endocrinol. Metab.* **43**, 831–835.
11. Tanaka, K., Nicholson, W. E. & Orth, D. N. (1977) *Front. Horm. Res.* **4**, 208–214.
12. Himsworth, R. L., Bloomfield, G. A., Coombes, R. C., Ellison, M., Gilkes, J. J. H., Lowry, P. J., Setchell, K. D. R., Slavin, G. & Rees, L. H. (1977) *Clin. Endocrinol.* **7**, 45–62.
13. Gewirtz, G., Schneider, B., Krieger, D. T. & Yalow, R. S. (1974) *J. Clin. Endocrinol. Metab.* **38**, 227–230.
14. Orth, D. N. & Nicholson, W. E. (1977) *J. Clin. Endocrinol. Metab.* **44**, 214–217.
15. Orth, D. N., Guillemin, R., Ling, N. & Nicholson, W. E. (1978) *J. Clin. Endocrinol. Metab.* **46**, 849–852.
16. Pettengill, O. S., Maurer, L. H. & Faulkner, C. S. (1977) *Proc. Am. Assoc. Cancer Res.* **18**, 121 (abstr.).
17. Sorenson, G. D. & Brinck-Johnsen, T. (1977) *Proc. Am. Assoc. Cancer Res.* **18**, 248 (abstr.).
18. Orth, D. N. (1978) in *Methods of Hormone Radioimmunoassay*, eds. Jaffe, B. M. & Behrman, H. R. (Academic, New York), 2nd Ed., in press.
19. Guillemin, R., Ling, N. & Vargo, T. M. (1977) *Biochem. Biophys. Res. Commun.* **77**, 361–366.
20. Orth, D. N., Tanaka, K. & Nicholson, W. E. (1978) in *Methods of Hormone Radioimmunoassay*, eds. Jaffe, B. M. & Behrman, H. R. (Academic, New York), 2nd Ed., in press.
21. Konigsberg, W. (1972) in *Methods in Enzymol.*, eds. Hirs, C. H. W. & Timasheff, S. N. (Academic, New York), Vol. 25, pp. 185–188.
22. March, S. C., Parikh, I. & Cuatrecasas, P. (1974) *Anal. Biochem.* **60**, 149–152.
23. Gospodarowicz, D. (1972) *J. Biol. Chem.* **247**, 6491–6498.
24. Tanaka, K., Nicholson, W. E. & Orth, D. N. (1978) *J. Clin. Invest.* **62**, 94–104.
25. Liddle, G. W., Givens, J. R., Nicholson, W. E. & Island, D. P. (1964) *Excerpta Med. Intern. Congr. Ser.* **83**, 1063–1067.
26. Cohn, D. V., Macgregor, R. R., Sinha, D., Huang, D. W. Y., Edelhoch, H. & Hamilton, J. W. (1974) *Arch. Biochem. Biophys.* **164**, 669–673.
27. Fish, W. W., Mann, K. G. & Tanford, C. (1969) *J. Biol. Chem.* **244**, 4989–4994.