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Immunological evidence for two common precursors to corticotropins, endorphins, and melanotropin in the neurointermediate lobe of the toad pituitary

(β-lipotropin/opiate peptides/*Xenopus laevis*/immunoprecipitation)

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ABSTRACT **The biosynthesis of corticotropin ($ACTH_{1-39}$), β-endorphin [β(61–91)-lipotropin] and α-melanotropin in the toad intermediate lobe was studied by using immunoprecipitation procedures with antisera specific for these peptides. Intermediate lobes were pulse-incubated with [^{3}H]phenylalanine and then chase-incubated for varying periods; the radioactive proteins were immunoprecipitated. Immunoprecipitates were separated by acidic urea or sodium dodecyl sulfate polyacrylamide gel electrophoresis. Evidence from the pulse–chase and sequential immunoprecipitation studies using antisera to ACTH and β-endorphin suggests that the toad intermediate lobe synthesizes two common precursors (apparent M_r 32,000 and 29,500) containing both the ACTH and β-endorphin sequences. These precursors are processed to yield several forms of immunoreactive corticotropin (apparent M_r 23,000, 21,000, 13,000, and 4300), immunoreactive endorphin (apparent M_r 11,700 and 3500), and immunoreactive α-melanotropin. The 4300 M_r form of corticotropin and the 11,700 and 3500 M_r forms of endorphins were found to comigrate with synthetic $ACTH_{1-39}$, β-lipotropin and β-endorphin, respectively, on both acidic urea and sodium dodecyl sulfate gels.**

A number of peptide hormones and neuroactive peptides are synthesized by sequential cleavages from larger precursors (1–3) and are models for post-translational control in protein biosynthesis. Among such peptides are a set of structurally related molecules, corticotropin (ACTH), β-lipotropin (β-LPH) and α-melanotropin (α-MSH) which are found both in the pituitary and in brain of lower vertebrates and mammals (4–6). Fragments of ACTH ($ACTH_{1-10}$, $ACTH_{4-10}$, and α-MSH) have been found to have central nervous system effects (7) whereas fragments of β-LPH [β-endorphin (β-LPH_{61-91}), α-endorphin (β-LPH_{61-70}), and enkephalin (β-LPH_{61-65})] appear to have opiate-like activity (8–10). The recent implications of the physiological significance of these peptides in the nervous system stimulated great interest in their biosynthesis, particularly in determining if they are cleaved from ACTH and β-LPH. Furthermore, the finding of larger forms of ACTH with immunoreactive and bioassayable corticotropic activity led to the hypothesis that ACTH itself may be a cleaved product of a larger precursor (11, 12).

Direct evidence for this hypothesis was first reported by Mains *et al.* (13) who discovered that pituitary tumor cells (line AtT-20) from mouse appear to synthesize not only ACTH but also β-LPH from cleavage of a common precursor of approximately 31,000 daltons. In addition, a polyadenylylated RNA fraction from such tumor cells has been translated in the reticulocyte and wheat germ cell-free systems and, among the translation products, is a 28,500-dalton protein that is precipitated by anti-ACTH antisera and includes tryptic digestion sequences that are indistinguishable from those of ACTH and β-endorphin (14, 15). At present, there is no report in the literature dealing with the issue of whether these biosynthetic and processing pathways occur in normal, intact tissues.

This paper presents the results of biosynthesis studies in the intermediate lobe of the toad (*Xenopus laevis*), which synthesizes ACTH, β-LPH, and α-MSH (16). The data show that this tissue synthesizes two common precursors that are immunoreactive with antibodies to ACTH and β-endorphin. These precursors (M_r 32,000 and 29,500) are processed to ACTH, β-LPH, β-endorphin, and α-MSH. Although the major cleaved products appear to be similar to those of the mouse AtT-20 tumor cell line, there are more cleavage products found in the intact intermediate lobe. These include α-MSH and a previously unreported endorphin-like peptide. Moreover, unlike the AtT-20 tumor cell line which releases the precursor molecules, the intermediate lobe does not release the precursors and it secretes only the processed peptides.

MATERIALS AND METHODS

Animals. Adult toads (*X. laevis*) weighing 40–70 g were purchased from NASCO Biological Supplies (Fort Atkinson, WI) and maintained in plastic aquaria at 22°C with dark/light cycles. Before use in experiments, animals were placed on a black background and dark-adapted for 15–30 days.

Incubation of Neurointermediate Lobes in [^{3}H]Phenylalanine. Neurointermediate lobes were dissected from the toads and preincubated at 22°C in amphibian Ringer's solution (NaCl, 112 mM; KCl, 2 mM; $CaCl_2$, 2 mM; Hepes, 15 mM; glucose, 5 mg/ml; bovine serum albumin, 1 mg/ml; ascorbic acid, 1 mg/liter; pH 7.35) for 1 hr to maximize MSH peptide biosynthesis *in vitro* (17). The neurointermediate lobes were then pulse-incubated in the presence of 19.2 μM [^{3}H]phenylalanine [New England Nuclear, Boston, MA; specific activity, 13.5 Ci (1 Ci = 3.7×10^{10} becquerels)/mmol] for 4 or 0.5 hr. After the pulse, the neurointermediate lobes were homogenized. A group of 0.5-hr pulsed lobes were subjected to a chase in amphibian Ringer's solution containing unlabeled phenylalanine (1 mM) for 1 or 3 hr before homogenization. The medium after a 3-hr chase was also collected for analysis of released peptides. Lobes were homogenized in 5 M acetic acid containing 5 mg of bovine serum albumin per ml and 1% phenylmethylsulfonyl fluoride (30 mg/ml)/iodoacetamide (30 mg/ml) in absolute ethanol, freshly made within hours of its use. Samples were frozen and thawed three times on dry ice and extracted at 4°C for 12–16 hr. Extracts were then lyophilized.

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Abbreviations: LPH, lipotropin; ACTH, corticotropin; MSH, melanotropin; $NaDodSO_4$, sodium dodecyl sulfate.

Control experiments using autoradiography at the light microscopic level (unpublished data) showed that the isotope penetrated throughout the cells in the intact intermediate lobe after 0.5 hr of incubation. After the pulse, most of the silver grains were localized over the intermediate lobe rather than the neural lobe; the latter was totally devoid of grains after the 1-hr chase period. Thus, the labeled peptides under study are synthesized in the intermediate lobe.

Immunoprecipitations. A double-antibody immunoprecipitation scheme was used exactly as described by Mains and Eipper (18) except that buffer E contained 1% Triton X-100. Purified anti-α-$ACTH_{1-24}$ (Bertha) antiserum was prepared as described (18). Rabbit antiserum to synthetic β-endorphin (RB100) was specific for the COOH terminus of β-LPH (beyond residue 77, and did not crossreact with α-endorphin) (19). The α-MSH antiserum was specific for the COOH-terminal peptide (Lys-Pro-Val-NH_2) of α-MSH because minal cross-reactivity was observed with $ACTH_{1-10}$, $ACTH_{4-10}$, $ACTH_{1-24}$, and $ACTH_{1-39}$, whereas $ACTH_{1-13}NH_2$ showed 70% cross-reactivity (20). The goat antiserum to rabbit immunoglobulins, used to precipitate the antibody–peptide complex, was purchased from Research Products Inc. (Elk Grove, IL).

Analysis of [^{3}H]Phenylalanine-Labeled Immunoprecipitates. The washed immunoprecipitates were dissolved in 0.9 M acetic acid/10 M urea overnight at 22°C, and were then electrophoresed on 10% polyacrylamide/acid/urea gels, pH 2.7 (21). When the M_r of the peaks separated on the acidic gels were to be determined, the labeled peptides on the unstained gel were cut out, eluted with sodium dodecyl sulfate (NaDodSO$_4$) sample buffer and reelectrophoresed on NaDodSO$_4$ by a procedure described elsewhere (22). Some immunoprecipitates were analyzed directly on NaDodSO$_4$ gels. The M_r of the peak running with synthetic β-endorphin on acid/urea gels was also determined by gel filtration on a Sephadex G-50 column in 6 M guanidine hydrochloride (23).

RESULTS

Extensive pulse–chase isotope labeling studies in previous work (16) suggested that the toad intermediate lobe synthesized a R_F 0.46 precursor (analyzed on acid/urea gels) that was processed to form melantropically active peptides that ran with ACTH, β-LPH, and α-MSH. The present study aimed at showing the immunological relationships of this precursor and other processed peaks with ACTH, β-LPH, and α-MSH, by immunoprecipitation of the [^{3}H]phenylalanine-labeled peptides (synthesized after a pulse or a chase) with specific antisera to these peptides. The acid/urea gel profiles of peptides immunoprecipitated with purified α-$ACTH_{1-24}$ antiserum are shown in Fig. 1. After a 30-min pulse, the dominant component was the precursor peak "a_1" (see Table 1 for mobility and M_r), and a second significant peak, "a_2," also was observed. A 30-min pulse followed by a 1-hr chase in the presence of 1 mM unlabeled phenylalanine resulted in a significant decrease in radioactivity in peak "a_1", and peak "a_2" almost disappeared. Concomitantly, other forms of ACTH-related peptides appeared: peaks "c" (R_F 0.65), "e" (R_F 0.83), and "i" (R_F 1.38), with apparent M_rs of 21,000, 13,000, and 4300, respectively (Table 1). Peak "i" ran with synthetic $ACTH_{1-39}$ on acid/urea gels and had a M_r similar to that of $ACTH_{1-39}$ on NaDodSO$_4$ gels. Previous work (16) showed that the processed products are maximally released after a 3-hr chase. Immunoprecipitation of the 3-hr chase medium with anti-α-$ACTH_{1-24}$ antiserum showed that peaks "c," "e," and "i" were released but peaks "a_1" and "a_2" were not (data not shown).

Fig. 2 shows the acid/urea gel profiles of [^{3}H]phenylalanine-labeled peptides immunoprecipitated by β-endorphin antiserum (RB100). As in Fig. 1, the major β-endorphin-related peak seen after a 30-min pulse was the precursor "a_1"; the hump (peak) "a_2" also was present. With a 1-hr chase in the presence of unlabeled phenylalanine, peak "a_2" disappeared and there was a significant decrease in radioactivity in peak "a_1." Peaks "g" and "m" which ran with β-LPH and β-endorphin, respectively, on acid/urea gels were formed. In addition, the synthesis of another peptide with endorphin-like immuno-

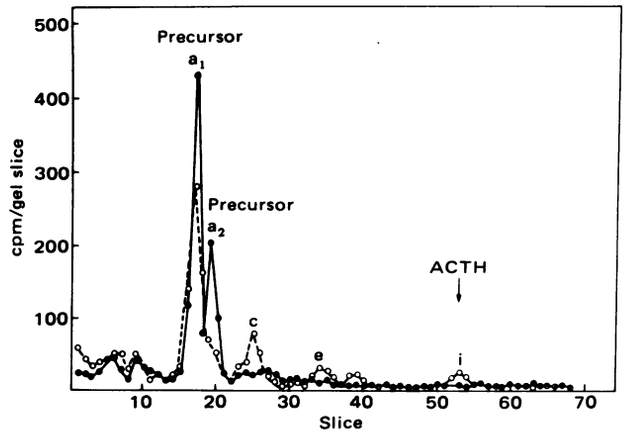

FIG. 1. Immunoprecipitation of intermediate lobe peptides with ACTH antiserum. Pituitary intermediate lobes were pulse labeled with [^{3}H]phenylalanine for 30 min (●—●) or pulsed for 30 min followed by a 1-hr chase in medium containing unlabeled phenylalanine (O - - - O). Each labeled lobe was homogenized and then immunoprecipitated with anti-α-$ACTH_{1-24}$ antiserum; the immunoprecipitates were analyzed on acid/urea gels. Arrow, mobility of synthetic $ACTH_{1-39}$.

Table 1. [^{3}H]Phenylalanine-labeled peptides synthesized in the toad intermediate lobe immunoprecipitated by specific antisera to ACTH, β-endorphin, and α-MSH

Immunoprecipitated peak	R_F	Apparent M_r	Antiserum to: ACTH	β-Endorphin	α-MSH	Released*
"a_1"	0.46–0.47	32,000	+	+	−	0
"a_2"	0.52–0.54	29,500	+	+	−	0
"b"	0.56–0.58	23,000	+	−	−	0
"c"	0.65–0.67	21,000	+	−	−	+
"e"	0.81–0.83	13,000	+	−	−	+
"g"	0.99–1.01	11,700	−	+	−	+
"i"	1.38–1.41	4,300	+	−	−	+
"j"	1.65–1.72†	<3,000	−	−	+	+
"m"	1.50–1.52	3,500	−	+	−	+

The immunoprecipitated peaks are illustrated in Figs. 1–3. R_F represents the mobility of each peak on acid/urea gels relative to cytochrome *c*. M_r was determined by elution of peaks from acid/urea gels and subsequent electrophoresis on NaDodSO$_4$ gels and by direct application of immunoprecipitates to NaDodSO$_4$ gels and correlation of the peaks with the acid/urea gel profile of the same aliquot. The M_r of peak "m," which falls close to the limit (≤3000) of accurate determination on NaDodSO$_4$ gels, was further confirmed by gel filtration on a Sephadex G-50 column in 6 M guanidine hydrochloride.

* +, Peptides released into the medium; 0, not released.

† The R_F of peak "j" was more variable than that of the other peaks; however, "j" consistently ran with synthetic α-MSH and methyl green.

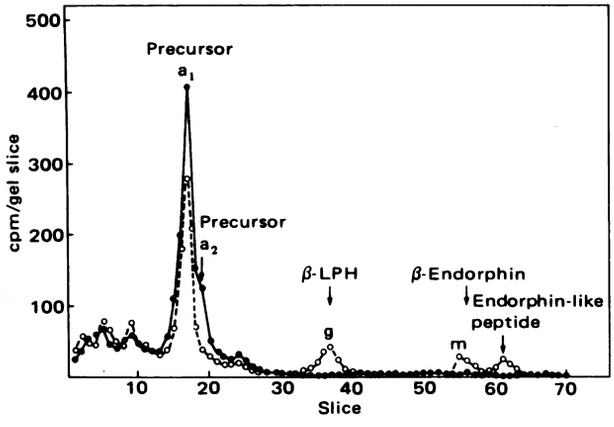

FIG. 2. . Immunoprecipitation of intermediate lobe peptides with endorphin antiserum. Intermediate lobes were labeled with [^{3}H]-phenylalanine and subjected to chase, as described in Fig. 1. The acid/urea gel profiles of immunoprecipitates after a 30-min pulse (●—●) and after a pulse followed by a 1-hr chase (O- - -O) are shown. The R_F of each peak is shown in Table 1. Arrows, mobilities of β-LPH and β-endorphin.

reactivity was observed. Immunoprecipitates of the medium collected after a 3-hr chase showed that peaks "g" (β-LPH), "m" (β-endorphin), and the endorphin-like peptide are released but the precursor peaks "a_1" or "a_2" are not (data not shown).

The pulse–chase data presented in Figs. 1 and 2 are consistent with the interpretation that various forms of ACTH, β-LPH, and β-endorphin are cleaved from larger precursors of R_F 0.46 and 0.52. To determine if the precursors of β-endorphin and ACTH are identical moelcules, sequential immunoprecipitations of 4-hr [^{3}H]phenylalanine-labeled lobes were carried out with antisera to ACTH and β-endorphin. Virtually no radioactivity in peaks "a_1" or "a_2" was precipitated with the second antibody (Table 2). These results indicate that peaks "a_1" and "a_2" are immunoprecipitated by both ACTH and β-endorphin antisera (Figs. 1 and 2). The data are consistent with their being common precursors for the synthesis of ACTH, β-LPH, and β-endorphin-related peptides.

Fig. 3 shows acid/urea gel profiles of 4-hr [^{3}H]phenylalanine-labeled peptides immunoprecipitated with antisera to ACTH, β-endorphin, and α-MSH. In Fig. 3A, immunoprecipitation using purified antiserum against α-ACTH$_{1-24}$ showed peaks "a_1," "a_2," "c," "e," and "i" in the profile, as in Fig. 1. In addition, a peak "b" was detected. Peak "b" represents another form of ACTH (R_F 0.58, M_r 23,000); it appears to have a rapid turnover and is not always detectable with a short pulse. However, peak "b" accumulated to a detectable level with long pulses. This peak has never been detected in the chase medium and is probably not released. When excess ACTH was added to the sample before immunoprecipitation, no radioactive peaks

Table 2. Sequential immunoprecipitations of [^{3}H]phenylalanine-labeled peptides with antisera to ACTH and β-endorphin

	cpm in immunoprecipitated peak			
	First experiment		Second experiment	
Peak	First antibody (endorphin)	Second antibody (ACTH)	First antibody (ACTH)	Second antibody (endorphin)
"a_1"	202	17	198	15
"a_2"	61	16	70	16

Two aliquots, consisting of 1/100th of a 4-hr [^{3}H]phenylalanine-labeled lobe were immunoprecipitated with either the ACTH or the β-endorphin antiserum. The supernatants from these immunoprecipitates were then immunoprecipitated with the second antiserum. All four immunoprecipitates were analysed on acid/urea gels. The amount of label in the two precursor peaks "a_1" and "a_2" are shown.

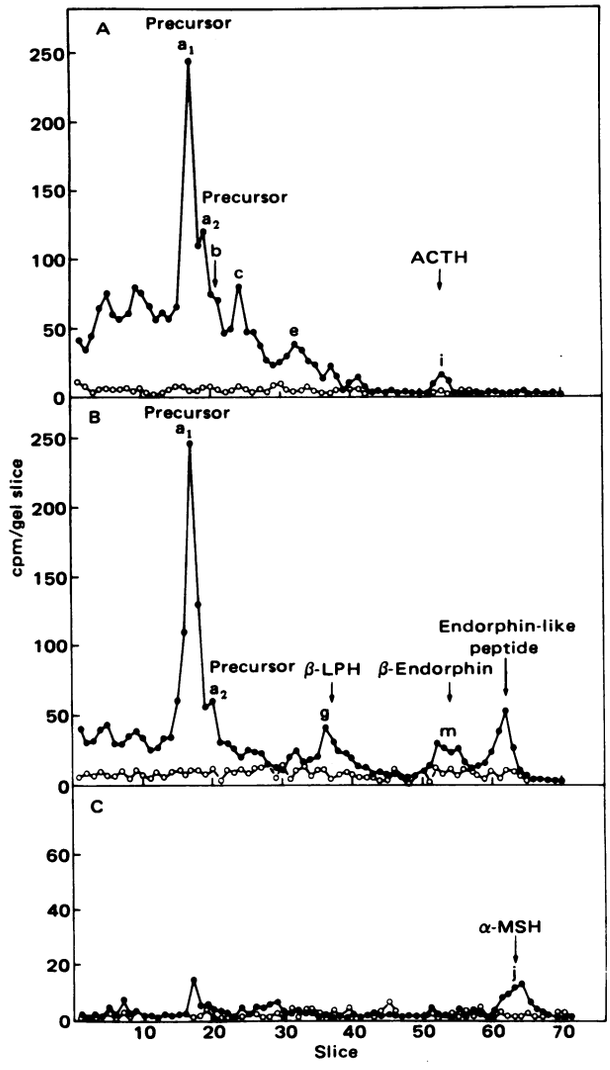

FIG. 3. Intermediate lobes were pulse-labeled with [^{3}H]phenylalanine for 4 hr and the radioactive peptides were immunoprecipitated with ACTH (*A*), β-endorphin (*B*) or α-MSH (*C*) antiserum. The immunoprecipitates were analyzed on acid/urea gels. The R_F and molecular weight of each of the major immunoprecipitated peaks in each of the profiles are summarized in Table 1. Radioactivity in each gel slice was assayed to <5% error. (*A*) Immunoprecipitates of an aliquot of the labeled lobe with anti-α-ACTH$_{1-24}$ antiserum (●—●); and another aliquot with excess (5 μg) synthetic ACTH$_{1-39}$ added before immunoprecipitation with ACTH antiserum (O- - -O). (*B*) Immunoprecipitates of an aliquot of the labeled lobe with β-endorphin (RB100) antiserum (●—●); and another aliquot with excess (5 μg) synthetic β-endorphin added before immunoprecipitation with β-endorphin (RB100) antiserum (O- - -O). (*C*) Immunoprecipitates of an aliquot of the labeled lobe with α-MSH antiserum (●—●); and another aliquot with excess (5 μg) synthetic MSH added before immunoprecipitation with MSH antiserum (O- - -O). The radioactivity was low because the specific activity of the labeled α-MSH in the lobe was low and there is a limit on the amount of protein (peptide + specific antiserum + goat anti-rabbit immunoglobins) that can be loaded on the gel. However, the gel pattern was highly reproducible.

were detectable. Thus, the antiserum is specific for ACTH-related peptides because binding to the radioactive molecules can be completely prevented with excess unlabeled ACTH.

Fig. 3*B* shows the various peptide peaks ("a_1," "a_2," "g," and "m") immunoprecipitated by antiserum to β-endorphin, in 4-hr pulsed lobes. A significant amount of endorphin-like peptide also was present after a long pulse compared to the 30-min pulse–1-hr chase experiments (Fig. 2). Peak "b" shown in Fig. 3*A* did not appear to be precipitated by β-endorphin antiserum. The immunoprecipitation of the radioactive β-endorphin-related peptides was blocked by excess β-endorphin, showing the specificity of the antiserum.

Fig. 3*C* shows the results when a similar aliquot of [^{3}H]-phenylalanine-labeled lobe was immunoprecipitated with antiserum specific for the COOH-terminal sequence of α-MSH. Consistent with the specificity of the antiserum, there was little crossreactivity with precursors "a_1" and "a_2" and the other forms of ACTH, but a peak "j" which runs with synthetic α-MSH was observed. Pulse–chase studies showed that peak "j" was present only after a 1-hr chase following a 30-min pulse (16). Thus, α-MSH is probably cleaved from one or both of precursors "a_1" and "a_2". Specificity of binding of the antiserum to α-MSH is shown by the lack of immunoprecipitation of radioactive peak "j" in the presence of excess synthetic α-MSH.

DISCUSSION

Pulse–chase experiments with different isotopes (arginine, phenylalanine, methionine), followed by a bioassay for melanotropic activity (16) and immunoprecipitation with specific antisera (figures 1–3 in ref. 24; unpublished methionine data) to identify the labeled peptides, demonstrate that the toad intermediate lobe synthesizes two common precursors* (a major peak "a_1," and a minor peak "a_2"; see Figs. 1 and 2 and Tables 1 and 2) of ACTH and β-endorphin. Similar results have been found by Roberts and Herbert (14) in the AtT-20 mouse anterior pituitary tumor line, where three common precursors (M_r 34,000, 32,000, and 29,000) of ACTH and β-endorphin were synthesized, of which the 32,000 M_r species was the major component. The precursors (peaks "a_1" and "a_2") are processed to four major forms of ACTH-related peptides (see Figs. 1–3 and Table 1): peaks "b," "c," "e," and "i." The ACTH form denoted by peak "i" has been identified as $ACTH_{1-39}$ because it runs with synthetic $ACTH_{1-39}$ on acid/urea and $NaDodSO_4$ gels and is melanotropically active (6, 16). This $ACTH_{1-39}$ (peak "i") may in turn be cleaved to yield α-MSH (peak "j") which is observed only after a chase of 1 hr, at which time ACTH (peak "i") is also present (16). Peak "j" correlates with the R_F of a band of strong bioassayable melanotropic activity extracted from toad intermediate lobe and separated on acid/urea gels (6, 16). Peak "b" appears to be a short-lived intermediate of processing, whereas peaks "c," "e," "i," and "j" are products that are released by the lobe. Studies reported elsewhere (24) have shown that the precursors "a_1" and possibly "a_2", as well as peaks "c" and "e," are glycosylated.

* Peaks "a_1" and "a_2" of this paper are contained within peak "a" described in previous publications (16, 24). The radioactivity, after a pulse, in peaks "a_1" and "a_2" immunoprecipitated with either ACTH or β-endorphin antiserum is 75–80% of that in the nonimmunoprecipitated peak "a." Thus, the presence of nonprecursor proteins within peak "a" of a nonimmunoprecipitated sample is rather small. Peak "a_2" is reflected within the broad descending phase of peak "a" and cannot be easily identified as a separate component without immunoprecipitation. The migration of nonprecursor proteins between peaks "a_1" and "a_2" may account for the lack of resolution of the minor peak "a_2" in a nonimmunoprecipitated sample.

Processing of the common precursors "a_1" and "a_2" also yields another set of peptides related to β-endorphin: peaks "g" and "m" and an endorphin-like peptide. Peak "g" which runs with β-LPH and peak "m" which runs with synthetic β-endorphin, on both acid/urea and $NaDodSO_4$ gels, have been identified as β-LPH and β-endorphin, respectively. Peak "m" was not observed when lobes were pulse labeled with [^{3}H]arginine (unpublished data). This suggests that the β-endorphin synthesized by the toad intermediate lobe is fairly similar to pituitary β-endorphin in other species, which also does not contain arginine (9). β-LPH (peak "g") and β-endorphin (peak "m") are in the medium after a 3-hr chase. Although only the precursors and major processed products have been focused on, close examination of the gel profiles (Figs. 1–3) suggests that there may be other minor peaks present. In particular, there appears to be two slowly migrating peaks [mobility < peak "a," M_r 100,000–200,000 estimated by the Ferguson (26) analysis on acid/urea gels (24, 25)] immunoprecipitated by ACTH and β-endorphin antisera. These are unlikely to be precursors because they are present during a 30-min pulse and do not appear to be processed with chase. Clearly, the system is very complex.

Comparison of the ACTH/β-endorphin precursors and processed products synthesized by the toad intact intermediate lobe with those synthesized by the AtT-20 mouse anterior pituitary cell line and rat pituitary cell suspensions (27) reveal great similarity. Mains *et al.* (13) showed that the AtT-20 cells synthesize a glycosylated common precursor (M_r 31,000) that is processed to two glycosylated ACTH forms (M_r 23,000 and 13,000), $ACTH_{1-39}$ (M_r 4,500) (16, 21), β-LPH (M_r 11,700) and β-endorphin (M_r 3500). The presence of more forms of ACTH- and β-endorphin-related peptides in the toad intermediate lobe may be due to extra cleavage steps (e.g., in the formation of α-MSH from ACTH) or to better resolution by using acid/urea gels as compared to $NaDodSO_4$ gels used by the investigators of the AtT-20 cells. One significant difference between the intact intermediate lobe and the AtT-20 tumor line is that, whereas no precursors (peak "a_1" and "a_2") are released in the former, the latter releases the M_r 31,000 precursor. A plausible explanation is that the toad intact intermediate lobe has the ability to regulate the release of newly formed granules, with a time delay such that, when they are released, all the precursor molecules within the granules have been processed. Evidence from subcellular fractionation studies in the toad intermediate lobe suggests that the precursors are packaged within the granules and processing occurs intragranularly (unpublished data). The AtT-20 tumor cells may not have this regulating capacity or, alternatively, may be releasing the M_r 31,000 precursor (not packaged in granules) by leakage through the plasma membrane.

The significance of having two common precursors is unclear. Possibly, different sets of processed peptides are cleaved from each of the precursors [e.g., the glycosylated ACTH forms may be derived from the more glycosylated precursor (peak "a_1"), and the unglycosylated forms, from the other]. Perhaps some peptides (e.g., β-LPH and β-endorphin) may be cleaved from both precursors. The kinetics suggest that the cleavages occur rapidly, and perhaps several peptides may be cleaved simultaneously. Amino acid sequence studies and carbohydrate analysis will be necessary to resolve the questions of (*i*) whether the primary sturcture of the precursors are identical, the only variant being in the degree of glycosylation, as has been suggested by work on the AtT-20 cell line (28, 29) and (*ii*) whether the peptides are cleaved from one or both precursors. Investigations into the significance of the carbohydrate on the common precursors with an inhibitor of glycosylation (tunicamycin)

suggest that the sugars play an important role in stabilizing these molecules and in the processing (24). The physiological significance of simultaneously releasing multiple peptides with different functions (corticotropins versus endorphins) is an interesting problem which awaits further study. Perhaps some of the glycosylated forms of ACTH may have longer half-lives in the blood, because the carbohydrate appears to have a stabilizing effect (24) and thus prolongs the action of these peptides.

Note Added in Proof. Since this manuscript was submitted, it has been shown that cell suspensions from anterior and neurointermediate lobes of the rat pituitary have a biosynthetic pathway for ACTH, β-LPH, and β-endorphin similar to that of the toad neurointermediate lobe (27). One significant difference is that, whereas the 21,000 M_r form of ACTH represents a major biosynthetic and release product in the toad, it is a minor component in the rat intermediate lobe. It has also been proposed from recent studies on the AtT-20 tumor cell line that the common precursors for ACTH and β-endorphin differ in the degree of glycosylation (28, 29).

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