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Glucocorticoid Inhibition of Immunoreactive β-Endorphin Release from the Anterior Lobe of the Rat Pituitary: *In Vitro* and *in Vivo* Studies (42106)

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Abstract. Glucocorticoid control of pituitary β-endorphin (β-END) release was investigated *in vitro* and *in vivo*. Cultured cells of both rat anterior (AL) and neurointermediate (NIL) lobe released β-END-like immunoreactivity (β-END-LI) in response to epinephrine (10^{-7} *M*); however, only the response of AL cells was prevented by corticosterone (10^{-8}–10^{-6} *M*) or dexamethasone (10^{-9}–10^{-7} *M*). Gel chromatographic analysis (Sephadex G-50) revealed that the major forms of β-END-LI released by AL cells corresponded to β-END and β-lipotropin (β-LPH) in molecular size, whereas virtually all of the immunoreactivity released by NIL cells resembled β-END. *In vivo* administration of dexamethasone attenuated the stress-induced release of β-END-LI in a dose- and time-related fashion, having a more pronounced effect on plasma levels of β-END-LI corresponding to β-LPH in molecular size. Metyrapone (100 mg/kg), an inhibitor of glucocorticoid synthesis, evoked a rapid (20–40 min) four- to sixfold increase in total plasma β-END-LI and 75% of this rise was due to immunoreactivity resembling β-LPH in size. This response was diminished by coadministration of either dexamethasone (0.05–1.25 mg/kg) or corticosterone (0.05–1.25 mg/kg) and completely prevented by 4-hr pretreatment with dexamethasone (50 μg/kg). The briskness of the plasma β-END-LI response to acute changes in glucocorticoid status suggests that a "rapid" feedback mechanism operates in the physiologic control of pituitary β-END-LI secretion. Moreover, the ability of glucocorticoids to selectively inhibit AL release of β-END-LI *in vitro* and their pronounced effect on plasma levels of β-END-LI resembling β-LPH, a marker of AL secretion, together indicate that glucocorticoids exert a selective influence over the secretion of AL corticotrophs *in vivo*. This demonstration of differential regulation of the AL versus IL secretion of β-END-LI *in vivo* most likely reflects a phenomena having biologic importance related to the different physiologic actions of the several molecular forms of β-END-LI secreted by the two tissues.

Recent *in vitro* findings indicate that separate mechanisms control the *in vivo* secretion of β-endorphin-like immunoreactivity (β-END-LI) by corticotrophs of the anterior lobe (AL, pars distalis) and melanotrophs of the intermediate lobe (IL, pars intermedia) of the rat. For example, corticotropin-releasing factor (CRF) profoundly stimulates the release of β-END-LI from primary cell cultures of AL, yet, has comparatively minor effects on β-END-LI release by IL cells (1–4). Similarly, the direct inhibitory actions of glucocorticoids on pituitary β-END-LI release are restricted to the AL over a lower dose range (1, 2, 5–7). On the other hand, dopamine appears to be an important inhibitor of release from the IL but it has no direct effect on AL secretion (1, 8, 9). Although epinephrine directly stimulates β-END-LI release from AL as well as IL cells, it appears to do so through separate receptor mechanisms; an α-adrenoceptor mediates epinephrine-induced release from the AL, whereas a β-adrenoceptor operates on the IL (1, 2, 6, 8–11). These differences which clearly distinguish the *in vitro* release of β-END-LI from AL versus IL cells suggest that separate mechanisms govern secretion from the two lobes *in vivo*.

The physiologic importance for different mechanisms regulating AL versus IL secretion of β-END-LI may relate to the different biologic actions of the molecular forms of β-END-LI produced by the two tissues. Based on tissue content and *in vitro* release studies (12–16; see Fig. 1), it appears that the principal forms of immunoreactive β-END secreted by the AL *in vivo* are $\beta\text{-END}_{1\text{–}31}$ and β-lipotropin (β-LPH), whereas the IL produces primarily shorter and/or acetylated

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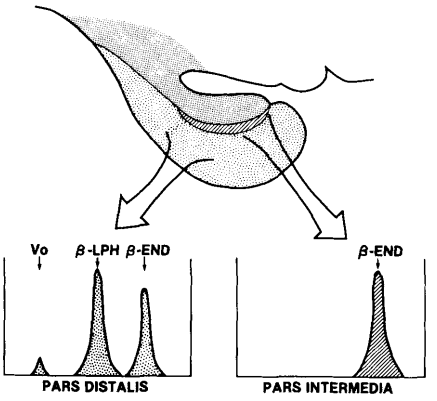

FIG. 1. A schematic representation of the rat pituitary showing the different molecular weight forms of β-END-LI secreted by the pars distalis and pars intermedia *in vitro*. The chromatograms represent gel filtration elution profiles (Sephadex G-50) of β-END-LI release by cell cultures of the two lobes. Corticotrophs of the pars distalis are distinguished from melanotrophs of the pars intermedia by the secretion of higher molecular weight forms of β-END-LI, principally β-LPH. By contrast, virtually all of the immunoreactivity released by melanotrophs corresponds to β-END in molecular size. Adapted from (6, 9, 12–16) and findings presented here.

forms of β-END. Of these peptides, only β-END_{1-31} appears to function as an opiate receptor ligand (16) and biologic actions for β-LPH and the β-END peptides of the IL remain to be established. Although not yet defined, likely differences in the biologic actions of the β-END forms produced by the AL and IL suggest a need for differential regulation of the two lobes *in vivo*.

In this report we compare the distinctive gel chromatographic profiles of immunoreactive β-END released by AL and IL cells *in vitro* with those present in rat plasma. When interpreting changes in plasma levels of β-END-LI resembling β-LPH to reflect release from the AL, findings presented here indicate that glucocorticoids selectively and rapidly regulate the physiologic release of β-END-LI from the AL. Further, the predominant form of β-END-LI secreted by the AL *in vivo* appears to be β-LPH. Taken together, these findings also indicate that physiologic stimuli and pharmacologic agents can be used in conjunction with *in vitro* methods, gel filtration chromatography, and radioimmunoassay analysis for studying the differential control of the AL and IL *in vivo*.

Materials and Methods. *Animals, in vivo treatments, and sample collection.* Male, Sprague–Dawley rats (Taconic Farms, Inc., Germantown, N.Y.) weighing 150 to 200 g and having free access to standard rat chow (Charles River, Syracuse, N.Y.) were housed with lights on from 0600 to 1800 hr. To limit the effects of nonspecific stress, all animals used in the *in vivo* experiments were accustomed to daily handling for at least 3 days before experimentation. Further, animals were housed in the experimental environment for 24 hr prior to treatment. Dexamethasone sodium phosphate (Carter-Glogau Labs, Glendale, Ariz.) or metyrapone (Ciba Pharmaceutical Co., Summit, N.J.) was diluted in 0.9% NaCl and administered by either subcutaneous or intraperitoneal injection. Corticosterone (Sigma Chemical Co., St. Louis, Mo.) was dissolved in absolute ethanol, diluted to appropriate final concentrations in 0.9% NaCl, and injected (0.2 ml/100 g body wt) intraperitoneally; the final concentration of ethanol was 5%. Control animals received appropriate vehicle injections. Rats subjected to physical immobilization were loosely taped to test tube racks and placed on their backs; 20 min later they were freed and blood samples collected. This procedure provides reproducible stimulus for pituitary β-END-LI release which is maximal by 10 to 30 min (12, 13) yet does not physically injure the animal. Other treatment times as well as drug doses are given under Results. After decapitation (between 0900 and 1300 hr) blood samples were collected into plastic tubes containing 0.5 ml 10% ethylenediamine tetraacetic acid in 0.05 *M* phosphate buffer (pH 7.2) and kept on ice. Plasma samples were separated by centrifugation and stored at −70°C until chromatographed or assayed.

Tissue culture and in vitro release. Primary cell cultures of rat AL and IL were established as previously described (17). The AL and IL were dissected separately as the anterior or combined neurointermediate (NIL) lobes, respectively. Following enzymatic dissociation, approximately 7×10^4 AL or NIL cells were plated (10×35-mm culture dishes, Falcon,

Oxnard, Calif.) in 2 ml culture medium and grown at 37°C under 95% air and 5% carbon dioxide. The culture medium consisted of Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, N.Y.) containing 10% horse serum (Gibco) and 2.5% fetal calf serum (Gibco), 0.1 m*M* glutamine, and 1% nonessential amino acids (Gibco). Release experiments with AL and NIL cells were carried out 6 and 8 days, respectively, after plating. Cultures were washed four times with 2 ml Eagle's modified essential medium (Gibco) containing 2% horse serum and then exposed to various test substances in 2 ml incubation medium (DMEM containing 2% horse serum). L-Epinephrine (Sigma) was dissolved in water containing 0.5 mg/ml ascorbic acid and subsequently diluted with incubation medium to a final concentration of 10^{-7} *M;* a concentration we have determined to increase β-END-LI release from cultures of both AL and NIL cells to a similar extent (17). Corticosterone (Sigma) or dexamethasone (Sigma) were dissolved in absolute ethanol and diluted with incubation medium to the final concentrations indicated under Results. The concentrations of ascorbic acid and ethanol to which all plates were exposed were 50 ng/ml and 0.01%, respectively. Epinephrine and/or dexamethasone treatments were for 2 hr; corticosterone was added to the plates 4 hr prior to sample collection. Incubation media of the dexamethasone experiment included bacitracin (30 μg/ml), whereas that of the corticosterone experiment did not. The presence of the protease inhibitor had no effect on the absolute amount or the chromatographic profile of the β-END-LI released. Samples were stored at −70°C until chromatographed or assayed.

Gel filtration chromatography. Samples of pooled plasma (6–8 ml) or pooled incubation media (5–7 ml) (taken from representative treatment groups) were chromatographed on a Sephadex G-50 (medium, fine, or superfine, Pharmacia Inc., Piscataway, N.J.) column (2.5 × 80 cm) and eluted (0.5 ml/min, 7.0-ml fractions) at 4°C with 0.1 *N* acetic acid containing 0.05% bovine serum albumin, 0.02% sodium azide, and 0.03 mg/ml bacitracin (Sigma). Aliquots (3 ml) of each fraction were lyophilized and resuspended in assay buffer (600 μl) for assay of β-END-LI. Recoveries of applied β-END-LI (determined for each run) ranged from 70 to 90% (N = 10); all data presented are corrected to 100% recovery. Variations in recovery reflect differences due to the lyophilization and reconstitution procedures. Repeated analysis of the same sample (N = 10) resulted in a range of recoveries (71–95%) for total β-END-LI which was equally accounted for in each peak. Camel $\beta\text{-END}_{1\text{-}31}$, α-*N*-acetyl-$\beta\text{-END}_{1\text{-}31}$, α-*N*-acetyl-$\beta\text{-END}_{1\text{-}27}$, $\beta\text{-END}_{1\text{-}2}$ (Peninsula, San Carlos, Calif.), and purified human β-LPH (A. Parlow and D. Orth) and rat β-LPH (prepared in house) were used as standards. Due to its smaller size, rat β-LPH migrates one to two fractions behind human β-LPH in this chromatographic system (18).

Radioimmunoassay. Levels of β-END-LI in plasma, incubation media, and gel filtration elution profiles were estimated by radioimmunoassay (RIA) as previously described (17, 19). At a final dilution of 1:125,000, the antiserum employed (C-55, raised in a rabbit against camel $\beta\text{-END}_{1\text{-}31}$) binds 30–35% of iodinated β-END in the absence of unlabeled peptide and the RIA is sensitive to 10 pg camel $\beta\text{-END}_{1\text{-}31}$ standard (Peninsula). Purified human β-LPH (A. Parlow and D. Orth) as well as the free and *N*-acetylated forms of camel $\beta\text{-END}_{1\text{-}31}$ and camel $\beta\text{-END}_{1\text{-}27}$ and α-*N*-acetyl-$\beta\text{-END}_{1\text{-}26}$ (Peninsula) are detected on an equimolar basis. The antiserum does not recognize, however, peptides related to the N-terminal amino acid sequence of β-END (i.e., methionine and leucine enkephalin, α-endorphin) or nonopiate hypothalamic, pituitary, or gastrointestinal hormones. Dose–response curves of plasma, incubation medium, and gel filtration fractions paralleled camel $\beta\text{-END}_{1\text{-}31}$ standard.

Statistics. Statistical differences between groups ($P < 0.05$) were determined by analysis of variance and Duncan's multiple range test.

Results. Epinephrine (10^{-7} *M*) stimulated the release of β-END-LI by cultures of both AL and NIL cells (Fig. 2). The presence of corticosterone in the incubation medium (10^{-8}–10^{-6} *M*) caused a dose-related attenuation of the epinephrine-induced release of β-END-LI from AL cultures but did not affect the enhanced secretion from NIL cells.

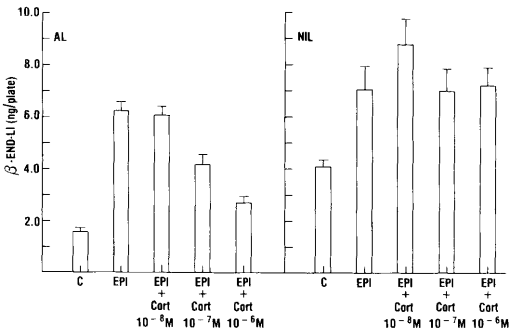

FIG. 2. Dose–response effects of corticosterone on the epinephrine-induced release of β-END-LI by cultured anterior lobe (AL) and neurointermediate lobe (NIL) cells. Cells were incubated with epinephrine (EPI, 10^{-7} *M*, 2 hr) alone or in combination with corticosterone (Cort, 2-hr preincubation followed by 2-hr incubation) at the concentrations indicated. Bars and vertical lines represent group means ± SE (N = 5–6 plates per group). Corticosterone at 10^{-7} and 10^{-6} *M*, but not 10^{-8} *M*, significantly attenuated ($P < 0.05$) the epinephrine-induced release of β-END-LI from AL cells; all doses of corticosterone were without effect on β-END-LI release by NIL cells.

Basal release of β-END-LI by AL or NIL cells was not significantly affected by corticosterone at the doses tested (10^{-8}–10^{-6} *M*; data not shown). The effects of dexamethasone (10^{-9}–10^{-7} *M*) on basal and epinephrine-stimulated release of β-END-LI by AL and NIL were comparable to those observed for corticosterone (data not shown). Figure 3 shows the chromatographic profiles of β-END-LI released by AL cells exposed to control medium, or medium containing epinephrine (10^{-7} *M*) alone or in combination with corticosterone (10^{-6} *M*). Under control conditions, AL cells released two major forms of β-END-LI corresponding to β-END_{1-31} and β-LPH in molecular size. In addition, small amounts (less than 10% of total eluted) of a high molecular weight form eluting near the void volume and a species which coeluted with the β-END_{1-27} standards (free and α-*N*-acetylated) were also detected; the latter form was positioned on the declining side of the β-END_{1-31} peak. Epinephrine stimulated the release of all molecular forms of immunoreactivity produced by AL cells and corticosterone counteracted the responses of β-END-LI eluting at the void volume and in the regions of β-LPH and β-END_{1-31} standards. The minimal epinephrine-induced rise observed in β-END-LI resembling β-END_{1-27} was not clearly influenced by corticosterone (or dexamethasone, data not shown) suggesting that this material may have been released by a small number of pars intermedia cells which could have contaminated the dissection of the AL (see introduction). In contrast to AL cultures, NIL cells only released forms of β-END-LI which corresponded to β-END_{1-31} and β-END_{1-27} standards and no immunoreactivity resembling β-LPH or higher molecular weight proteins were detected in the incubation media of the different

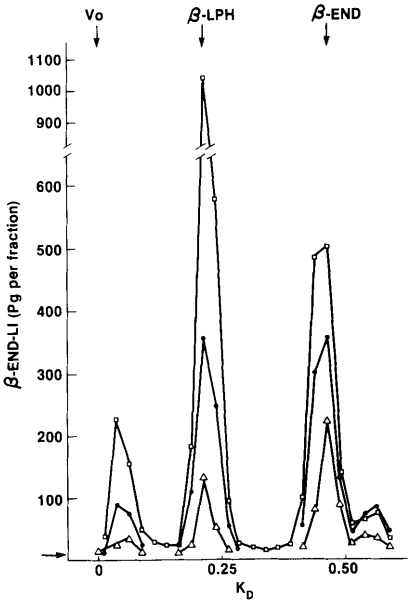

FIG. 3. Comparison of gel chromatographic profiles of β-END-LI released from AL cells incubated in control medium (△) or medium containing epinephrine (10^{-7} *M*) (□) or epinephrine (10^{-7} *M*) plus corticosterone (10^{-7} *M*) (●). Pooled (6 ml) release media from groups presented in Fig. 2 were chromatographed (Sephadex 50, superfine). The elution positions for the void volume (V_0), and β-LPH and β-END_{1-31} standards are indicated across the top; assay sensitivity (10 pg) is indicated by the arrow on the ordinate. Data are representative of two chromatographic studies using samples from two experiments. Further, comparable results were obtained from experiments (2) in which epinephrine (10^{-7} *M*) and dexamethasone (10^{-7} *M*) were used as treatments.

treatment groups. Consistent with earlier reports (1, 10), neither corticosterone (10^{-8}–10^{-6} M) nor dexamethasone (10^{-9}–10^{-7} M) altered basal or stimulated release of β-END-LI from NIL cells (data not shown). Thus, the secretion of β-END-LI corresponding to β-LPH and inhibition of this release by glucocorticoids distinguishes the *in vitro* secretion of β-END-LI by cells of the AL versus IL.

Presented in Fig. 4 are the effects of dexamethasone, physical immobilization, and the two treatments in combination on the chromatographic profile of circulating β-END-LI in rats. In this experiment dexamethasone treatment tended to lower basal levels of total plasma β-END-LI (0.15 ± 0.02 ng/ml vs 0.12 ± 0.02 ng/ml; not significant) and dramatically inhibited the 11-fold increase in plasma β-END-LI content caused by immobilization (1.64 ± 19 ng/ml vs 0.47 ± 0.05 ng/ml, $P < 0.05$). The elution profile of plasma collected from control animals revealed two peaks of β-END-LI, a higher molecular weight form which cochromatographed with purified β-LPH standards represented 30% of the total eluted β-END-LI; the remaining 70% corresponded to β-END standards in molecular size. Physical immobilization increased the size of both peaks having a more pronounced effect on β-END-LI resembling β-LPH. Pretreatment with dexamethasone reduced the β-LPH form of plasma β-END-LI to below the limit of detection (10 pg/200 μl resuspension) in both nonstressed and immobilized rats. As compared to the profile of plasma β-END-LI from control rats, dexamethasone treatment alone had no noticeable effect on the size or symmetry of the peak eluting in the region of β-END but did, however, reduce by 50% the increase in this peak caused by restraint stress.

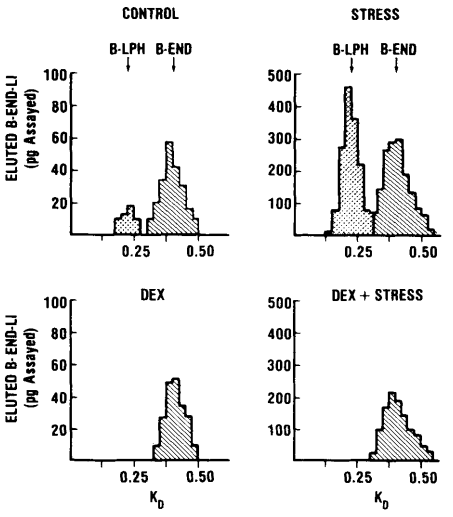

FIG. 4. Gel chromatographic profiles of plasma β-END-LI; effects of dexamethasone and physical immobilization. Pooled plasma samples (6 ml) from animals treated with either vehicle (CONTROL), dexamethasone (DEX, 300 μg/kg, 8 days, sc) or physical restraint (STRESS, 20 min) were chromatographed (Sephadex, G-50, medium). Elution positions of β-LPH and $\beta\text{-END}_{1\text{-}31}$ standards are indicated above the upper chromatograms. Void volume is $K_D = 0$ and the limit of assay sensitivity was 10 pg $\beta\text{-END}_{1\text{-}31}$. Levels of total plasma β-END-LI for the treatment groups were CONTROL = 0.15 ± 0.02 ng/ml, DEX = 0.12 ± 0.02 ng/ml, STRESS = 1.64 ± 0.19 ng/ml, and DEX + STRESS = 0.47 ± 0.05 ng/ml. Data are representative of six chromatographic studies using samples from six experiments.

To further examine the effects of dexamethasone on the different chromatographic forms of circulating β-END-LI, a second dose–response experiment was conducted. In Fig. 5 the subdivisions of each bar show the effects of increasing doses of dexamethasone pretreatment on the relative contributions of β-LPH size and β-END size forms of immunoreactivity to total plasma β-END-LI in physically immobilized rats. Confirming and extending the findings presented in Fig. 4, stress alone increased plasma concentrations of both forms of β-END-LI having a somewhat greater influence on that resembling β-LPH. The contribution of the β-LPH-like material to total plasma β-END-LI was increased from 29 to 43% after 20-min immobilization. Pretreatment with 30 μg/kg dexamethasone (8 days) produced a significant reduction ($P < 0.05$) in the stress-induced increase in total plasma β-END-LI (2.28 ± 0.33 ng/ml vs 1.24 ± 0.30 ng/ml) and reduced the contribution of β-END-LI resembling β-LPH to 16%. In these same animals the dexamethasone treatment had no apparent effect on the amount of circulating β-END-LI resembling β-END in size. A higher dose of dexamethasone (100 μg/kg)

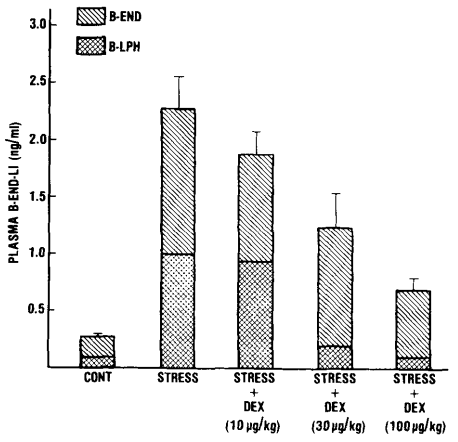

FIG. 5. Dose–response effects of dexamethasone on the stress-induced release of β-END-LI. Dexamethasone (DEX)-treated animals received 8 daily sc injections of 10, 30, or 100 μg/kg, stressed animals were immobilized for 20 min. Each bar represents the group mean ± SE (N = 7) of total plasma β-END-LI. Plasma pools (6.0 ml) of each treatment group were chromatographed (Sephadex G-50, fine) and the subdivisions of each bar indicate the relative contributions of β-END and β-LPH forms of immunoreactivity to total plasma β-END-LI. Data are representative of eight chromatographic studies using samples from eight experiments.

further decreased total plasma β-END-LI in a manner which was reflected in both β-LPH and β-END forms.

The dose–response and time–course effects of metyrapone treatment on levels of total plasma β-END-LI are shown in Fig. 6. As compared to vehicle injected control values, a single intraperitoneal injection of metyrapone (100 mg/kg) maximally increased circulating β-END-LI by 20 to 40 min after injection (0.32 ± 0.05 ng/ml, vs 1.10 ± 0.16 ng/ml and 1.29 ± 0.19 ng/ml, respectively). By 180 min postinjection, plasma levels of β-END-LI had returned to control values. In contrast, metyrapone in doses of 10 and 30 mg/kg was without effect on blood content of β-END-LI by 40 min after injection. These observations indicating that the effects of metyrapone on plasma β-END-LI occur rapidly and over a narrow dose range are consistent with similar observations on the ability of metyrapone to inhibit corticosterone secretion (20, 21). It should also be noted that administration of metyrapone (100 mg/kg) produced an immediate and transient period (15 sec) of discomfort (abdominal pain accompanied by abdominal contraction) followed by sedation (decrease motor activity and listlessness) which was most pronounced 10 to 20 min after injection. These behavioral responses were not reversed by pretreatment with dexamethasone in doses which prevented the effect of metyrapone on plasma levels of β-END-LI (see Fig. 7). Coadministration of dexamethasone or corticosterone with metyrapone attenuated, by up to 50%, the increase in total plasma β-END-LI evoked by metyrapone alone (100 mg/kg, 40 min; Table I); 4-hr pretreatment with dexamethasone (50 μg/kg) completely prevented the metyrapone-induced increase in total plasma β-END-LI (Fig. 7, top panels). Chromatographic analysis of treatment group plasma pools (Fig. 7, bottom panels and subdivisions of bars) showed that 75% of the increase in total plasma β-END-LI following metyrapone was due to immunoreactivity resembling β-LPH

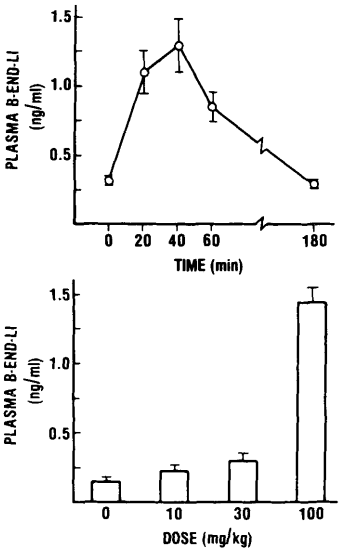

FIG. 6. Dose–response and time–course effects of metyrapone on levels of plasma β-END-LI. Groups of animals (N = 6–7) received either vehicle (0 min or 0 mg/kg) or metyrapone ip. The dose of metyrapone injected in the time–course study (top graph) was 100 mg/kg; blood samples were collected 40 min after injection in the dose–response experiment (lower graph). Data represent group means ± SE.

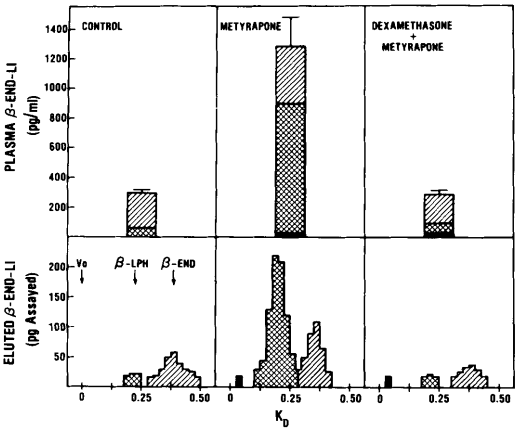

FIG. 7. Effects of metyrapone and dexamethasone on plasma β-END-LI levels and gel chromatographic profiles of plasma β-END-LI. Metyrapone (100 mg/kg, 40 min, ip) was given alone or following pretreatment with dexamethasone (50 μg/kg, 4 hr, sc). Levels of total plasma β-END-LI are shown in the top panels where bars and vertical lines represent group means ± SE (N = 6–7). Subdivisions of bars indicate the relative contributions to total plasma β-END-LI of immunoreactivity eluting in the regions of β-END_{1-31} (β-END), and β-LPH standards and at the void volume (V_0) (bottom panels). The different patterns of the subdivisions correspond to the peaks of β-END-LI in the chromatograms. Each chromatogram depicts the plasma elution profile (Sephadex G-50, fine) of a treatment group pool (6 ml). Data are representative of three chromatographic studies using samples from three experiments.

in molecular size. Immunoreactivity corresponding to β-END standard comprised almost all of the remaining portion with a trace of β-END-LI eluting at the void volume. Animals receiving dexamethasone pretreatment in combination with metyrapone had both plasma levels of total β-END-LI and chromatographic profiles which were similar to those observed in vehicle injected control rats.

Discussion. The present findings indicate that glucocorticoids selectively modify the *in vivo,* as well as *in vitro,* secretion of proopiomelanocortin-derived peptides from the AL of the rat. This example of differential regulation of AL versus IL secretion of β-END-LI presumably reflects a phenomena having physiologic importance related to the different biologic actions of the several molecular forms of β-END-LI secreted by the two tissues. Available evidence suggests that the β-END_{1-31} produced by the AL may function in endogenous pain suppression systems, whereas, the shorter and acetylated forms of β-END-LI released by the IL, and β-LPH produced by the AL, have actions which appear to be unrelated to opiate receptors and the perception of pain (12–16).

Both corticosterone and dexamethasone, in low doses, attenuate the direct stimulatory effect of epinephrine on β-END-LI release from dispersed cell cultures of AL but not IL cells. Importantly, epinephrine increases the release of all molecular forms of β-END-LI produced by AL and IL cells without altering qualitatively the distinctive chromatographic profiles of β-END-LI secreted by the two tissues. A major difference between the molecular forms of β-END-LI released by AL as compared to those of IL cells is the secretion of β-END-LI resembling β-LPH in

TABLE I. ACUTE DOSE-RESPONSE EFFECTS OF DEXAMETHASONE AND CORTICOSTERONE ON THE METYRAPONE-INDUCED INCREASE IN PLASMA β-END-LI

Treatment	Plasma β-END-LI (ng/ml)
Controls (vehicles)	0.19 ± 0.02
Metyrapone (100 mg/kg)	1.18 ± 0.13[a]
Metyrapone + dexamethasone (0.05 mg/kg)	0.76 ± 0.18[a,b]
Metyrapone + dexamethasone (0.25 mg/kg)	0.61 ± 0.10[a,b]
Metyrapone + dexamethasone (1.25 mg/kg)	0.58 ± 0.83[a,b]
Metyrapone + corticosterone (0.05 mg/kg)	0.81 ± 0.08[a,b]
Metyrapone + corticosterone (0.25 mg/kg)	0.87 ± 0.10[a,b]
Metyrapone + corticosterone (1.25 mg/kg)	0.56 ± 0.06[a,b]

Note. Drugs or vehicles were coadministered by ip injection; plasma samples were collected 40 min later. Each group contained six animals. Values are means ± SE.

[a] Values significantly different from the vehicle injected control mean ($P < 0.05$).

[b] Values of the metyrapone plus glucocorticoid-treated groups significantly different from the mean value of animals treated with metyrapone alone ($P < 0.05$).

molecular size (6, 9, 12–16; see Figs. 1 and 3). These *in vitro* observations suggest that changes in plasma levels of immunoreactivity corresponding to β-LPH reflect changes in the *in vivo* release of β-END-LI (and other biosynthetically related hormones) from corticotrophs of the AL. In support of this hypothesis we observed that *in vivo* administration of glucocorticoids and metyrapone predominantly effect circulating β-END-LI resembling β-LPH in molecular size. For example, a single injection of metyrapone increases total plasma β-END-LI fourfold and 75% of this rise is due to immunoreactivity which coelutes with rat β-LPH; pretreatment with dexamethasone completely prevents this response (Fig. 7). By contrast, dexamethasone and metyrapone have no effect on plasma levels of immunoreactive (α-MSH) which is cosecreted with β-END-LI by the melanotrophs of the IL (2, 7). These observations, therefore, further support the conclusion that glucocorticoids primarily affect the secretion of hormones from AL corticotrophs *in vivo*.

The briskness of the plasma β-END-LI response to metyrapone (Fig. 6) and glucocorticoids (Table I) indicates that a "rapid" feedback mechanism operates in the physiologic control of pituitary β-END-LI secretion. Although changes in plasma levels of corticosterone or adrenocorticotropin (ACTH) were not evaluated in the present study, Dallman *et al.* (22), Jones *et al.* (23) and others [see (24)] have demonstrated that rapid negative feedback by glucocorticoids is one of several components involved in the physiologic control of ACTH, another proopiomelanocortin-derived peptide secreted by AL corticotrophs.

Since the distinctive chromatographic profiles of β-END-LI released under basal conditions by cultured AL and IL cells, respectively, are not altered qualitatively under conditions of stimulated release [(6, 9, 12–16); Fig. 3], changes in the chromatographic profiles of plasma β-END-LI observed here are probably not due to alterations in the processing of β-LPH to β-END before release. It remains to be determined, however, the extent to which shifts in the chromatographic profiles of plasma β-END-LI may be related to experimentally induced changes in the clearance rates for the different forms of plasma β-END-LI. Since the half-lives of administered $\beta\text{-END}_{1\text{-}31}$ and $\beta\text{-LPH}_{1\text{-}91}$ have been reported to be quite short in rats (less than 10 min) (25, 26), it is possible that a relatively small change in the clearance rate for either β-END or β-LPH forms of plasma immunoreactivity could alter the chromatographic profile of circulating β-END-LI independent of a change in pituitary release. Nevertheless, the ability of glucocorticoids to selectively inhibit AL release of β-END-LI *in vitro* [Fig. 2 (1–3, 6, 22–24)] and their more pronounced effects on plasma levels of β-END-LI resembling β-LPH, a marker of AL secretion, together suggest that alterations in the release of pituitary β-END-LI is the major factor causing changes observed in the chromatographic profiles of circulating β-END-LI.

In contrast to glucocorticoids which affected primarily the fraction of plasma β-END-LI resembling β-LPH in molecular size, certain dopaminergic agonist drugs selectively decrease circulating levels of β-END-LI corresponding to β-END in size (together with α-MSH) [(27), Farah and Mueller, unpublished]. We interpret these findings to indicate that dopamine inhibits the release of β-END-LI from the IL *in vivo* as is the case *in vitro* (1, 8, 9). Although physical immobilization causes a greater relative increase in plasma β-END-LI resembling β-LPH, a dramatic rise in the β-END size form is also observed. Further, the proportional increase in the β-END peak is considerably more pronounced in restrained animals (Figs. 4 and 5) as compared to those treated with metyrapone (Fig. 7). Therefore, under conditions of physical immobilization a significant portion of total plasma β-END-LI appears to be derived from the IL. By contrast, the AL seems to be the primary origin of blood β-END-LI in animals treated with metyrapone. Consistent with this view, we have observed that plasma levels of α-MSH are increased ($P < 0.05$) by physical restraint but not metyrapone [(7) and unpublished]. Further, dopamine agonists were found to attenuate the stress-induced release of pituitary β-END-LI (28) yet have

no effect on the plasma β-END-LI response to metyrapone (29).

Interestingly, the effects of glucocorticoid status are most dramatically associated with the release of β-LPH from the AL. This finding strongly suggests physiological relevance for β-LPH in blood. Although actions of β-LPH on adrenal aldosterone secretion have been demonstrated both *in vitro* (30) and *in vivo* (31), other possible functions for β-LPH are open to question. Tissue analysis studies indicate that the smaller amounts of β-END-sized immunoreactivity coreleased with β-LPH from the AL corresponds to $\beta\text{-END}_{1-31}$. The ability of this peptide to interact with opiate receptors (16) predicts physiologic actions related to effects of morphine. These include possible functions in nociception, and gastrointestinal activity as well as effects on the immune system (32).

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