Glucocorticoid and Mineralocorticoid Effects on Adrenocorticotropin and β-Endorphin in the Adrenalectomized Rat

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ABSTRACT Immunoreactive ACTH (ir-ACTH) and immunoreactive β-endorphin (ir-βEP) were determined in plasma, anterior pituitary, neuro-intermediate lobe, and hypothalamus of sham-adrenalectomized rats, and adrenalectomized rats given six daily injections of vehicle (oil), dexamethasone, 9α-fluorocortisol or deoxycorticosterone. 6 d after adrenalectomy, anterior pituitary ir-ACTH and ir-βEP were double, and plasma levels approximately fivefold those in controls. Adrenalectomy did not alter hypothalamic levels of either peptide, or ir-βEP in neuro-intermediate lobe, in which tissue ir-ACTH was below detection limit at routine dilutions. Dexamethasone (0.2–200 μg/d) concurrently suppressed plasma ir-ACTH and ir-βEP, with a near maximal effect at 20 μg, and a half-maximal effect between 2 and 6 μg; similar dose-response characteristics were found for thymolysis. Step-wise increases in anterior pituitary content of both peptides were found, with no change in hypothalamic levels of either peptide, or neuro-intermediate lobe ir-βEP. 9α-fluorocortisol (0.2–200 μg/d) produced plasma, anterior pituitary, and hypothalamic effects equivalent to dexamethasone, but with one-tenth the potency. Unlike dexamethasone, higher doses of 9α-fluorocortisol significantly elevated neuro-intermediate lobe ir-βEP. Deoxycorticosterone (2–2,000 μg/d) produced no significant changes in plasma, anterior pituitary or hypothalamic levels of either peptide; like 9α-fluorocortisol, doses of >60 μg/d significantly elevated neuro-intermediate lobe ir-βEP. Whereas ir-ACTH and ir-βEP synthesis in and release from the anterior pituitary are under complex negative feedback glucocorticoid control, there exists a mineralocorticoid-specific effect on neuro-intermediate lobe content of ir-βEP.

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INTRODUCTION

Pituitary secretion of adrenocorticotropic hormone (ACTH) is influenced both by hypothalamic corticotropin-releasing factor (CRF)[1] and circulating glucocorticoids. In the rat, feedback inhibition of corticosteroids on ACTH release acts at both the hypothalamic and pituitary level (1). Under physiological conditions, hypothalamic CRF release is highly sensitive to inhibition by corticosterone, and thus CRF has been postulated as the major determinant of ACTH release (1). Administration of glucocorticoids to intact animals lowers plasma ACTH to varying degrees, depending on steroid potency, dose, and period of treatment (2, 3). Adrenalectomy removes the corticosteroid feedback inhibition, and increases both basal and CRF-stimulated secretion (1, 4). As a consequence, plasma levels and pituitary content of ACTH are concurrently elevated, the extent of change being related to the length of time postadrenalectomy (5, 6).

Cell-free messenger RNA translation and pulse-chase chromatographic studies have shown that β-endorphin (βEP) and ACTH share a common, 31K dalton precursor in the anterior pituitary (7), neuro-intermediate lobe (8), and hypothalamus (9), although the posttranslational processing of the 31K precursor varies between tissues (10, 11). There are some reports that plasma and pituitary immunoreactive (ir)-βEP, like ACTH, may be subject to glucocorticoid feedback inhibition; the area, however, remains one of some controversy. Adrenalectomy has been reported to re-

[1] *Abbreviations used in this paper*: αMSH, α-melanocyte-stimulating hormone; AP, anterior pituitary; β-EP, β-endorphin; CLIP, hACTH 18-39; CRF, corticotropin-releasing factor; CV, coefficient of variation; DM, dexamethasone; DOC, 11-deoxycorticosterone; HT, hypothalami; ir-βEP, immunoreactive βEP; N-IL, neuro-intermediate lobe; 9αfF, 9α-fluorocortisol; PBS, phosphate-buffered saline; RIA, radioimmunoassay.

duce pituitary immunoreactive β-endorphin (ir-βEP) (12); in contrast, long-term glucocorticoid treatment has also been reported (13) as reducing ir-βEP in both anterior pituitary and neuro-intermediate lobe (N-IL). However, normal levels of plasma βEP after dexamethasone have been reported both in monkeys and in patients with maintained suppression of plasma cortisol and, by inference, of ACTH (14).

Evidence of specific pituitary-mineralocorticoid interrelations is currently indirect and inferential. Recently, mineralocorticoid receptors have been demonstrated in rat anterior pituitary (15), and in GH_3D_6 pituitary tumor cells (16). Both α-melanocyte-stimulating hormone (αMSH; 17) and β-lipotropin (βLPH; 18), products of the 31K precursor, have been reported to stimulate the secretion of aldosterone from dispersed adrenal glomerulosa cells in culture. The possible effects of mineralocorticoids on secretion of ir-βEP or αMSH from the neuro-intermediate lobe have not been studied either in vivo or in vitro.

In the present study we have examined the effects of glucocorticoid and mineralocorticoid administration in vivo on levels of ir-βEP and ir-ACTH in anterior pituitary, neurointermediate lobe, hypothalamus and plasma. 1-d adrenalectomized and sham-adrenalectomized rats were injected intramuscularly with either maize oil (vehicle), dexamethasone, 9α-fluorocortisol, or 11-deoxycorticosterone daily for 6 d, over a range of doses. Tissue contents of ir-βEP and ir-ACTH were determined, and changes of body and organ weights recorded.

METHODS

Female Sprague-Dawley rats weighing 150–200 g, from a pathogen-free colony bred in the Central Animal House of Monash University, were used in all studies. β-endorphin (βLPH 61-91), α-endorphin (βLPH 61-76, αEP), γ-endorphin (βLPH 61-77, γEP), αMSH (ACTH 1-13), CLIP (hACTH 18-39), methionine-enkephalin (Met-enk) and leucine-enkephalin (Leu-enk) were purchased from Peninsula Laboratory (San Carlos, CA). Human ACTH 1-39 (MRC 74/555) was from the Medical Research Council (London, England); ACTH 1-24 (Synacthen) from Ciba-Geigy (Lane Cove, Australia); human βLPH 1-91 was the gift of Dr. L. Rees (St. Bartholomew's Hospital, London, England), and porcine βLPH 1-91 the gift of Dr. N. Ling (La Jolla, CA). Donkey anti-rabbit gamma globulin (RD17) was obtained from Burroughs Wellcome Ltd. (London, England). Radioactive ^{125}NaI was supplied by Australian Atomic Energy Commission (Lucas Heights, Australia), and porcine ^{125}I-ACTH 1-39 by International CEA (Gif-sur-Yvette, France). Dexamethasone (DM) and 11-deoxycorticosterone (DOC) were from Sigma Chemical Co. (St. Louis, MO); 9α-fluorocortisol (9αfF) was the gift of E. R. Squibb & Sons (Melbourne, Australia); EDTA and chloramine-T were from Merck AG (Darmstadt, West Germany); polyethylene glycol 6000 from British Drug Houses (Port Fairy, Australia); *N*-ethyl-maleimide and 1-ethyl-3(3-dimethylaminopropyl) carbodiimide, HCl from Calbiochem-Behring, Div. American Hoechst Corp. (La Jolla, CA); bovine serum albumin from Commonwealth Serum Labs (Melbourne, Australia), and Trasylol from Bayer Pharmaceuticals (Botany, Australia). All other reagents were from Ajax Chemicals (Sydney, Australia).

Bilateral adrenalectomy was performed by the dorsal midline approach under light ether anesthesia, and its completeness verified by inspection at the time of killing. Animals were allowed free access to rat chow, and water (preadrenalectomy) or 0.9% saline (postadrenalectomy). Rats were housed six per cage in an air-conditioned room with a controlled light-dark cycle (light 0600–1800 h). Each rat was numbered and weighed the day before the start of the experiment, and reweighed on the second to last day before its death. From 1 d postadrenalectomy, 0.1 ml of maize oil or steroids in maize oil were administered intramuscularly, daily between 0930 and 1030 for 6 consecutive d. Sham adrenalectomized rats were given oil. 4–6 h after the last injection, animals were guillotined in a separate room, within 2 min of being removed from their cages. DM and 9αfF were dissolved in 0.4 ml of ethanol, and subsequently diluted with maize oil, so that 0.1 ml contained 0.2, 0.6, 2, 6, 20, 60, or 200 μg; for DOC, concentrations were 20, 60, 200, 600, and 2,000 μg/0.1 ml.

Collection and processing of tissues. Immediately after killing, pituitary glands were carefully removed and separated into anterior pituitary (AP) and N-IL in chilled 0.1 N HCl under a dissecting microscope. Hypothalami were dissected according to the method of Iverson and Glowinsky (19). Tissues were then frozen at −20°C in 2 ml of 0.1 N HCl. From each animal the thymus gland, spleen, and kidneys were removed and weighed. Tissues for assay were extracted by boiling for 15 min, cooling on ice, and homogenization with a Polytron (Brinkmann Instruments, Inc., Westbury, NY, speed setting 2, 1 × 2-s burst). Homogenates were centrifuged (20,000 *g*, Sorvall RC-5, DuPont Instruments-Sorvall Biomedical Div., Newtown, CT) for 15 min at 4°C, and the supernatants removed and frozen in aliquots (−20°C), for radioimmunoassay (RIA). Trunk blood was collected in chilled heparinized tubes for βEP RIA, and into 1.5-ml Eppendorf microfuge tubes containing 50 μl of Trasylol, 2,100 Kallekrein inhibition units (KIU)/ml, *N*-ethylmaleimide 0.2 M, EDTA 0.05 M for ACTH RIA. After centrifugation (at 4°C) plasma was removed and frozen. The usual time interval between tissue processing and RIA was 7 d.

Radioimmunoassay of βEP and ACTH. Heterologous double antibody RIA systems were used for the estimation of both ir-βEP and ir-ACTH in tissue extracts and plasma. In the βEP RIA, synthetic human βEP was used both for standards and for radioiodination. Anti-βEP serum (R56) was raised in a rabbit immunized with ovine βEP conjugated to bovine serum albumin by 1-ethyl-3(3-dimethylaminopropyl) carbodiimide, HCl. The resulting protein-antigen conjugates were dialyzed, lyophilized, and subsequently emulsified with Freund's complete adjuvant. The emulsion was injected at 2–4-wk intervals into rabbits at multiple intradermal sites until a satisfactory antibody titer was obtained. Donkey anti-rabbit gamma globulin (RD17) was used as second antibody in the RIA system. ^{125}I-hβEP tracer was prepared by iodinating 1–3 μg hβEP with 1 mCi NaI^{125} in the presence of 10 μg chloramine-T for 30 s; the process was terminated by adding 10 μl of sodium metabisulphite, 3 mg/ml, in 0.01 M phosphate-buffered saline (PBS), 0.1% sodium azide, pH 7.5. The iodination mixture was loaded on a 1 × 15-cm Sephadex G-25 preequilibrated with PBS and coated with 2 ml of 2% bovine serum albumin. The iodination products were then

eluted with 0.1% gelatin in PBS, and 20 × 0.8-ml fractions collected. Specific activity of the tracer ranged between 150-300 μCi/μg; binding characteristics remained satisfactory for up to 5 wk of storage at −20°C.

The specific binding of ^{125}I-βEP to antiserum at a final dilution of 1:58,000 was 30%. Sensitivity, defined as the antigen concentration corresponding with the lower confidence limit of the number of counts specifically bound in the absence of unlabeled hormone, was 5±2.5 pg/tube (mean±SD, $n = 7$), representing 50 pg/ml assay sample. Nonspecific binding was routinely between 2 and 4.5%. The within assay coefficient of variation (CV), representing the variation of duplicates from the mean in six assays, was 8% at 125 pg/tube, and was <10% over the range of 20 to 200 pg/tube ($n = 6$). The between assay CV was 15% at 20 and 200 pg/tube ($n = 14$).

Synthetic human βEP and synthetic ovine βEP were equipotent in the assay. The anti-βEP serum does not recognize Met-enk, Leu-enk, ACTH 1-39, CLIP, αMSH, αEP, or γEP. The antibody, however, cross-reacts 50% on a molar basis with human βLPH and 100% with porcine βLPH. For assay, extracts of AP and N-IL in 2 ml 0.1 N HCl were diluted with βEP RIA buffer (0.5% bovine serum albumin in PBS with 0.02 M EDTA pH 7.4) to final concentrations between 1:2,000 and 1:5,000; hypothalami were diluted 1:50; plasmas were assayed either undiluted or 1:10 with RIA buffer, according to the doses of glucocorticoid administered. Sequential dilutions of tissue extracts and plasma showed good parallelism to the standard curve. Since nonparallelism in plasma samples was evident only when sample volumes exceeded 15% of the incubation volume, the sensitivity of the present RIA system allowed us to measure levels of βEP in intact rats (200–350 pg/ml) without plasma extraction. In each assay two standard curves and two quality control samples were included. All samples were assayed in duplicate at two dilutions.

For the ACTH RIA, antibody against Synacthen (ACTH 1–24) was raised in a rabbit (R1-3) in a similar manner as for hβEP. Iodinated porcine ACTH 1-39 was used as tracer, and human ACTH 1-39 as standard. Lyophilized ^{125}I-ACTH was reconstituted in 3 ml of Triton X-100 (1/100), 0.05 ml glacial acetic acid, and 2 ml acetone, and for assay use further diluted 1/50 with ACTH RIA buffer (0.05 M Tris, 0.0005 M Na_2EDTA, 200 KIU/ml Trasylol, 2% PEG 6,000, 0.01 M NEM, and 0.01% Triton X-100, 0.2% gelatin, pH 7.6). Binding characteristics remained satisfactory over 2 wk of storage at 4°C.

The specific binding of ^{125}I-ACTH to antiserum at final dilutions of 1:10,000 was 41%. Routine sensitivity of the RIA was 2±0.8 pg/tube (mean±SD, $n = 7$), with nonspecific binding 3%. The within assay CV was 8% at 250 pg/tube, and 12% over the range of 20–200 pg/tube ($n = 6$). The between assay CV was 14% at 200 pg/tube ($n = 12$). Synacthen (ACTH 1-24) and MRC standard human ACTH 1-39 (74/555) were equipotent in the assay; human CLIP (ACTH 18-39) crossreacted 0.15%, and porcine βLPH 0.03%. No displacement was found with ACTH 4-10, αMSH, synthetic human βEP or synthetic ovine βEP. Similar procedures as for tissue βEP extraction were adopted, except for trunk blood collection (see above). Tissue extracts were diluted appropriately with ACTH RIA buffer, and plasma was measured unextracted. For all samples, sequential dilution of tissue extracts showed good parallelism to the standard curve. A previously published computer program for RIA (20) was used to process both βEP and ACTH RIA data. Differences between experimental and control groups were evaluated by unpaired two-tailed Student's t tests. All values are expressed as mean, and standard error of the mean, unless stated otherwise.

RESULTS

Adrenalectomy and steroid administration. Effects on body weight and organ weights. The effect of adrenalectomy and steroid administration upon body weight and organ weights is shown in Fig. 1. In our

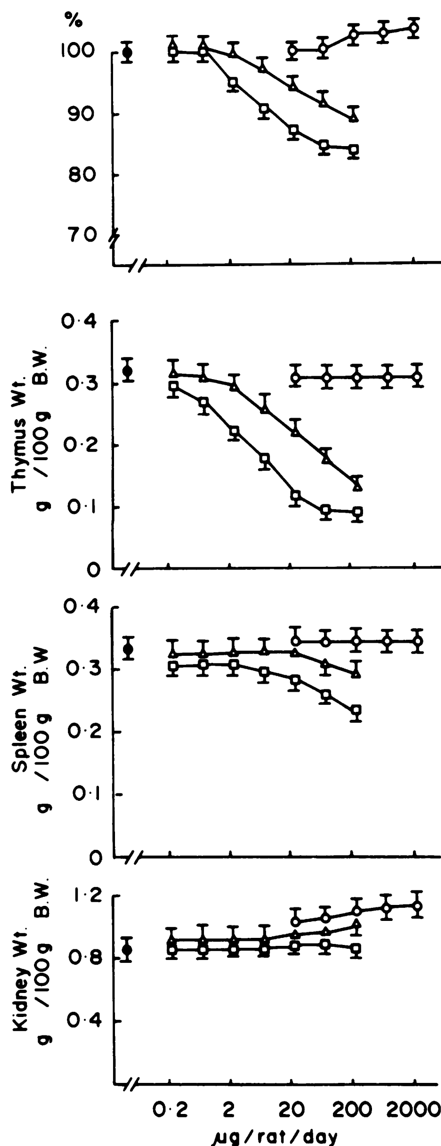

FIGURE 1 Effect of DM (□), 9αfF (△) and deoxycorticosterone (○) on body weight (B.W., top panel) and organ weights of adrenalectomized rats. Steroids or vehicle (0.1 ml maize oil: ●) were injected for 6 d; values for body weight are expressed as a percentage of control levels, and for organ weight as a fraction of body weight. For each point mean values±SEM are shown; $n = 6$.

study, the dose of DM needed for half-maximal thymolysis is between 2 and 6 μg/d, and that for splenic involution an order of magnitude higher. 9αfF causes similar thymic and splenic involution as DM, but at doses 10-fold higher; DOC appears without glucocorticoid effect in terms either of thymolysis or splenic involution.

Levels of ir-ACTH and ir-βEP. Effects of adrenalectomy. The effect of 6 d adrenalectomy on plasma and tissue levels of ir-ACTH and ir-βEP is shown in Fig. 2. Plasma levels of both peptides rose approximately fivefold above those found in sham adrenalectomized controls. Plasma ir-βEP levels in sham adrenalectomized animals ranged from 220 to 357 pg/ml (284±32, mean±SEM), levels similar to those previously found by others in unstressed rats killed at the same time of day as those in the present study (21).

Similarly, AP levels of both peptides rose after 6 d adrenalectomy to approximately double those in control, sham-adrenalectomized rats. In the hypothalamus, in contrast, no significant changes in ir-ACTH nor ir-βEP were seen; adrenalectomy similarly did not appear to alter neuro-intermediate lobe levels of ir-βEP. N-IL levels of ir-ACTH were universally below the sensitivity of the assay at the dilutions of tissue used.

Levels of ir-ACTH and ir-βEP. Effects of DM. In adrenalectomized animals, increasing daily doses of dexamethasone produced progressive reductions in plasma levels of ir-ACTH and ir-βEP, and a progressive elevation in anterior pituitary content of the two peptides (Fig. 3, upper panels). No differences between doses were seen in the hypothalamus or neuro-intermediate lobe (Fig. 3, lower panels).

The threshold dose for a suppressant effect, both on plasma ir-βEP and ir-ACTH, appears to lie between

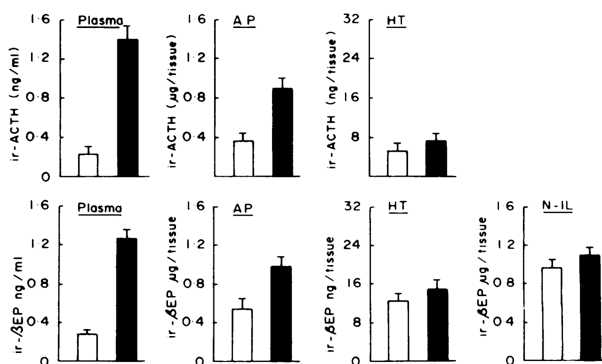

FIGURE 2 Effect of 6 d adrenalectomy (■) compared with sham adrenalectomy (□) on plasma and tissue levels of immunoreactive ACTH (upper panel) and immunoreactive β-endorphin (lower panel). All rats were injected with 0.1 ml i.m. maize oil daily; values shown are mean±SEM, $n = 6$.

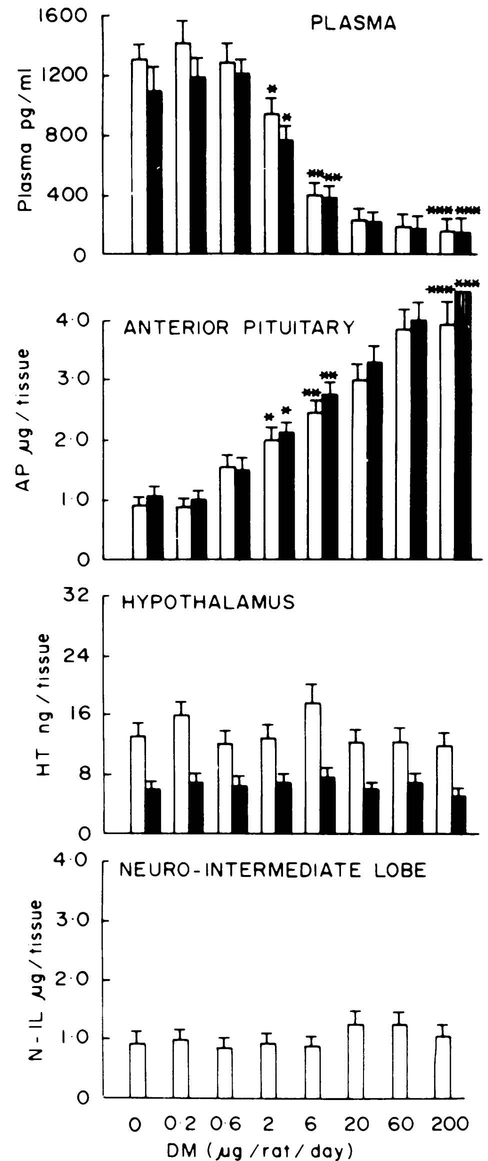

FIGURE 3 Effect of 6 d administration of DM on plasma and tissue levels of immunoreactive ACTH (■) and immunoreactive β-endorphin (□). Values shown are mean±SEM, $n = 6$. Compared with oil-injected controls, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

0.2 and 2.0 μg/d; the dose for a half-maximal effect between 2 and 6 μg/d; for both peptides, a plateau level, in terms of plasma suppression, is attained at 20 μg/d, with minimal further suppression at doses up to 200 μg/d.

In terms of the anterior pituitary content of ir-βEP and ir-ACTH, one similarity with the plasma profile is that the effects on ir-ACTH and ir-βEP appear closely coordinated in both tissues, strongly suggesting

parallel if not identical mechanisms regulating secretion and release of the two peptides.

There are, however, two differences between the dose-response curves for plasma and AP. First, the dose-response curve for AP ir-ACTH and ir-βEP appears to have a different shape to that for suppression of plasma levels of the two peptides; whereas the latter appear to be classic sigmoid dose-response curves, those for AP content show equivalent increments over a three-orders-of-magnitude dose range. Secondly, whereas increasing doses of DM progressively suppress plasma levels, they progressively elevate AP content of both peptides. This is in sharp contrast with previous reports of no change (22) or a reduction (13, 23) in AP content of ir-ACTH/ir-βEP after glucocorticoid administration. Such a finding presents an apparent paradox of an elevation in AP ir-ACTH and ir-βEP following adrenal steroid withdrawal, with a further increase after steroid replacement. Possible reasons for this discrepancy between our studies and those of other authors (13, 22, 23), and possible mechanisms of resolving the paradox, are discussed below.

Levels of ir-ACTH and ir-βEP. Effects of 9αfF. 9αfF is a potent synthetic mineralocorticoid, with considerable glucocorticoid activity in vitro and in vivo (24). The results of its administration to adrenalectomized rats are shown in Fig. 4. In terms of plasma and AP levels of ir-ACTH and ir-βEP, 9αfF appears to be ~10% as potent as DM, a finding consistent with its 10% thymolytic activity vis-a-vis DM. Like DM, 9αfF has no effect on hypothalamic levels of ir-ACTH and ir-βEP; in contrast with DM, however, the higher doses of 9αfF are followed by a significant elevation in N-IL content of ir-βEP (Fig. 4, bottom panel).

Levels of ir-ACTH and ir-βEP. Effects of DOC. DOC is a potent mineralocorticoid, with little or no agonist glucocorticoid activity in most test systems (24). The doses of DOC used in this study (up to 2,000 μg/d) produced no change in thymus weight, and no significant changes in plasma or AP levels of ir-ACTH and ir-βEP, comparing individual doses with control groups (Fig. 5, upper panels). Whether or not the apparent trend at high doses (suppression of plasma levels, increasing AP levels) represents minor partial agonist glucocorticoid activity, as has been shown for some glucocorticoid-inducible enzymes, cannot be answered with the range of doses used.

Like DM and 9αfF, DOC administration had no effect on hypothalamic levels of either peptide. Unlike DM—and consistent with the difference between DM and 9αfF—DOC administration elevates N-IL content of ir-βEP; as for 9αfF, the levels at >60 μg of steroid are significantly elevated (approximately twofold) over control (Fig. 5, lower panels).

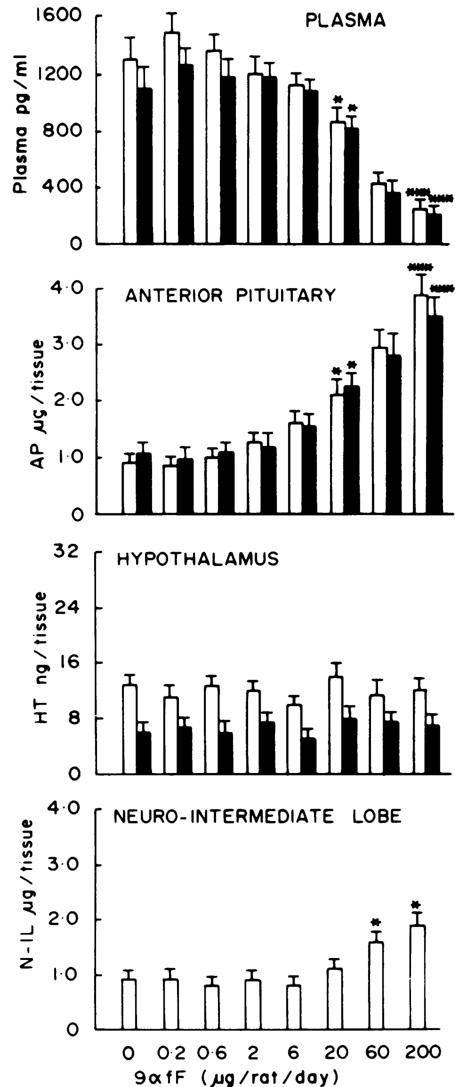

FIGURE 4 Effect of 6 d administration of 9αfF on plasma and tissue levels of immunoreactive ACTH (■) and ir-β-endorphin (□). Values shown are mean±SEM, $n = 6$. Compared with oil-injected controls, $^{*}P < 0.05$, $^{***}P < 0.001$.

DISCUSSION

The above findings appear worthy of discussion under several headings. First, anterior pituitary levels of ir-βEP and ir-ACTH double over a 6-d period after adrenalectomy; those in plasma are raised four- to sixfold after 6 d. These findings suggest that the rates of both precursor synthesis and release of ir-ACTH and ir-βEP from the anterior pituitary are increased, in response to the removal of glucocorticoid negative feedback inhibition, either directly or via enhanced CRF secretion. A corollary of the increase in both plasma levels and tissue content is that the increase in rate of syn-

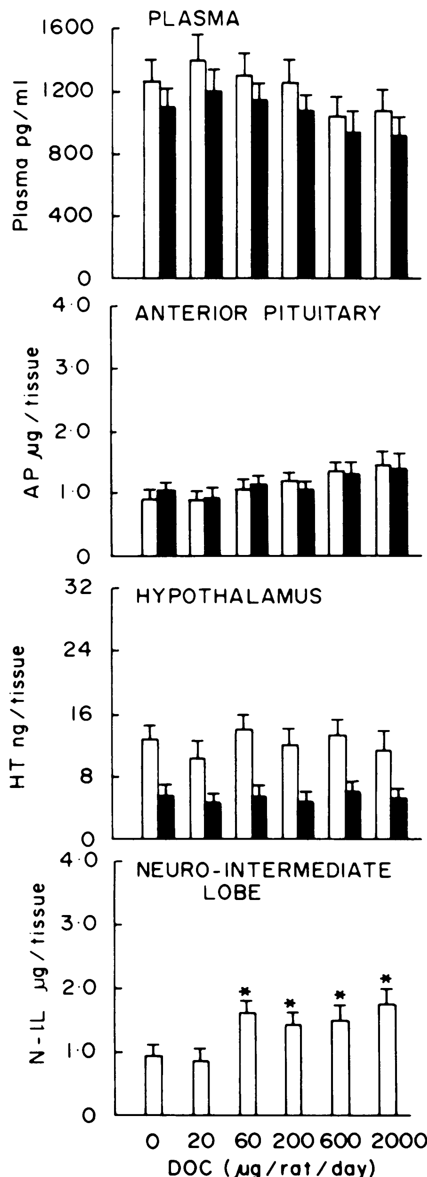

FIGURE 5 Effect of 6 d administration of DOC on plasma and tissue levels of immunoreactive ACTH (■) and immunoreactive β-endorphin (□). Values shown are mean±SEM, $n = 6$. Compared with oil-injected controls, $^{*}P < 0.05$.

thesis is at least marginally greater than the increase in rate of secretion, on the presumption that peripheral clearance is unchanged.

Such increases in plasma and AP content of ir-ACTH and ir-βEP postadrenalectomy are in general agreement with previous studies (13, 22, 25); exact comparisons of the magnitude of the changes are difficult in the light of differences in animal species, sex, age, and length of time postadrenalectomy. In contrast, although both reduced (12) and elevated (13) neurointermediate lobe contents of ir-βEP have been reported postadrenalectomy, our results substantiate neither observation. The present findings are, however, consistent with in vitro studies, in which the release of ir-βEP from N-IL in cell culture has been found to be influenced neither by CRF nor glucocorticoids (26).

Second, we have found that the contents of ir-βEP and ir-ACTH in the AP remain in a close stoichiometric relationship during various experimental manipulations, and that a similar relationship is also found in the plasma throughout the studies. The present findings are consistent with in vitro studies on AP showing that ir-ACTH and ir-βEP are derived from a common precursor, from which the two peptides are processed in a parallel manner; and that their concurrent release is modulated by both CRF and glucocorticoids (7, 8, 11, 13). The results, in addition, confirm previous studies (13) that ir-βEP and ir-ACTH are secreted concomitantly after adrenalectomy, and have extended this context to include that of glucocorticoid and mineralocorticoid treatment postadrenalectomy.

Third, plasma levels of ir-βEP and ir-ACTH are suppressed by DM in a dose-related manner, with the 50% effective dose (E_D50) between 2 µg and 6 µg, identical to the E_D50 for thymolysis. Although 20 µg of DM returns plasma ir-βEP and ir-ACTH levels to those found in the sham adrenalectomized animals, higher doses of DM do not significantly further lower this basal level. One possible interpretation is that the basal secretion rate of ir-βEP and ir-ACTH from the anterior pituitary is not under glucocorticoid negative feedback control; an alternative explanation would be that anterior pituitary secretion can be totally abolished by glucocorticoids, and that basal levels of ir-ACTH and ir-βEP are released from some other glucocorticoid insensitive tissue(s). The former appears the more plausible explanation, and consistent with the inability of glucocorticoids to suppress totally the secretion of ir-ACTH (27) or ir-βEP (26) from anterior pituitary cells in vitro.

Fourth, we have shown that DM elevates AP content of ir-βEP and ir-ACTH consistently and concurrently, in contrast with previous literature reports (2, 3, 13, 22, 25). The simplest interpretation of these data is that release of the peptides from the anterior pituitary is more sensitive to DM inhibition than is their synthesis. The doses of DM used in our studies were chosen to produce a full range of response in terms of inducing thymic involution and suppression of plasma ir-ACTH. The elevation in AP levels of both peptides, produced by a similar dose range of DM, is therefore potentially of physiological relevance. In marked contrast, previous studies (13, 22) have used much higher doses of DM (1.0–1.5 mg/rat per d). At such doses, even more marked body weight loss would be expected than was

found in the present studies. In the light of the profound catabolic state and presumably generalized inhibition of protein synthesis, reductions of AP ir-βEP or ir-ACTH reported in previous studies may reflect a nonspecific, toxic effect of DM.

Such differences in dose regime between studies do not, however, answer the question posed above; that of explaining how levels of AP ir-ACTH and ir-βEP in intact controls are lower than in either adrenalectomized or adrenalectomized-glucocorticoid-replaced animals. An answer may not be possible from existing data. We have measured ir-ACTH and ir-βEP; changes in precursor processing between intact, adrenalectomized, and adrenalectomized-steroid-replaced might explain the above finding at least in part, if significant differences in immunoreactivity exist between various precursor fragments; we have no quantitative data on the cross-reactivity of fragments larger than ACTH 1-39 or βLPH in our two assays. While changes in precursor processing may well occur, it seems unlikely that they make a substantial contribution to the observed phenomenon, given the strikingly coordinate changes in ir-ACTH and ir-βEP. In addition, preliminary gel chromatography studies show no discernible differences in AP ir-βEP elution position, between control animals and those injected with DM 200 μg; similarly, mineralocorticoids did not alter the elution position of N-IL ir-βEP (unpublished observation).

Two other additional, potentially complicating factors may be involved in the observed phenomenon. First, it is well established that glucocorticoids have both direct effects on pituitary secretion (1, 27), and indirect effects by altering CRF levels (28). Secondly, the feedback effects of glucocorticoids on ACTH are similarly not simple in terms of time (1, 28, 29). The present studies were at a single time point after adrenalectomy; AP levels of ir-βEP continue to rise for at least 32 d after adrenalectomy, and—in limited dose studies—continue to be further enhanced by DM 2 μg.[2] Our rats were killed 4–6 h after their last injection of steroid in oil. If AP levels are influenced by both fast and slow feedback, the sum of these two processes may be both time and dose dependent. Slow feedback would then show a dose-response curve similar to that for thymolysis; fast feedback would appear shifted to the right, in that only at higher doses of DM or the highest doses of 9αfF would there be adequate levels of circulating steroid immediately before killing.

A point that appears worthy of brief discussion is the content and relationship of ir-βEP and ir-ACTH in the hypothalamus. Unlike AP, ir-βEP content in HT is double that of ir-ACTH; both remain unchanged after both glucocorticoid and mineralocorticoid treatment. Recent immunohistochemical studies have shown that ir-αMSH coexists with ir-ACTH in hypothalamic neurons (30). Moreover, it has also been shown that a large proportion of ir-ACTH is processed into αMSH during axonal transport in HT cells (31). Since αMSH shows minimal cross-reactivity in our ACTH RIA, our findings of lower levels of ir-ACTH than ir-βEP are consistent with such processing; in addition, the maintained $\sim$1:2 ratio after adrenalectomy and during steroid treatment suggests that such processing is affected neither by glucocorticoids nor mineralocorticoids.

The other major finding of the study is that N-IL content of ir-βEP in adrenalectomized rats is elevated by mineralocorticoid, but not glucocorticoid administration. The changes are smaller in magnitude than the effect of DM on AP; they are induced with equivalent doses of 9αfF and DOC. The mechanisms whereby mineralocorticoids elevate N-IL ir-βEP is currently an area for speculation. High affinity, mineralocorticoid-specific aldosterone binding sites have recently been identified in rat anterior pituitary (15) and pituitary cell lines (16); we have consistently been unable to demonstrate such sites in rat N-IL preparations (Krozowski and Funder, unpublished data). If there are no mineralocorticoid receptors present in the N-IL, the effect of mineralocorticoids is presumably indirect, and perhaps most plausibly via altered neural input from the hypothalamic dopaminergic system. This would be consistent with in vitro studies, in which it has been shown that the release of N-IL ir-βEP is modulated by dopamine, but is unaffected by corticosteroids (26).

[2] Manuscript in preparation.

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