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Glucocorticoids regulate proopiomelanocortin gene expression *in vivo* at the levels of transcription and secretion

(solution hybridization/*in vitro* transcription/radioimmunoassay/adrenalectomy)

NEAL C. BIRNBERG*†, JEAN-CLAUDE LISSITZKY‡, MICHAEL HINMAN§, AND EDWARD HERBERT§

*Molecular Biology and Virology Laboratory, The Salk Institute for Biological Studies, San Diego, CA 92138; ‡Marseille University, Marseille, France; and §Department of Chemistry, University of Oregon, Eugene, OR 97403

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ABSTRACT **After adrenalectomy, the plasma levels of adrenocorticotropic hormone (corticotropin, ACTH)/endorphin peptides in rats rise dramatically in the first 4 hr while pituitary peptide levels fall sharply. Eight hours after adrenalectomy, plasma levels are near control values again but they then increase continuously over the next 8 days. Proopiomelanocortin (POMC) mRNA levels in the anterior pituitary (quantitated by hybridization with cloned POMC cDNA) increase 2-fold in the first 24 hours, reaching 15- to 20-fold the control level 18 days after adrenalectomy. When dexamethasone is administered to rats 8 days after adrenalectomy, the above events are reversed. Plasma ACTH falls to control levels within 2 hr whereas anterior pituitary POMC mRNA requires 5 days of treatment for return to control levels. The levels of POMC mRNA in the neurointermediate lobe and the hypothalamus are not altered by either treatment. Adrenalectomy increases transcription of the *POMC* gene in the anterior pituitary approximately 20-fold and halves transcription of the growth hormone gene within 1 hr of operation. Administration of dexamethasone immediately after adrenalectomy suppresses the increase in transcription of the *POMC* gene and increases the transcription of the growth hormone gene. Transcription of the *POMC* gene(s) in the neurointermediate lobe is not altered by either of these treatments.**

The gene(s) encoding proopiomelanocortin (POMC) in the mammalian neuroendocrine system is under complex hormonal control and is expressed in a tissue-specific manner (1–4). In the corticotrophic cells of the anterior pituitary, POMC is proteolytically processed to adrenocorticotropic hormone (corticotropin, ACTH), β-lipotropin, and amino-terminal peptide and secreted (5, 6). Secretion in these cells is thought to be negatively regulated by serum glucocorticoids and under positive control by hypothalamic corticotropin-releasing factor (CRF) (2, 7, 8).

The *POMC* gene(s) is also expressed in the neurointermediate lobe of the pituitary (9, 10) and hypothalamus (9). In the neurointermediate lobe, the *POMC* gene is expressed in virtually every cell and is thought to be under negative regulation via dopaminergic neurons descending from the hypothalamus (11). The primary secretion products from the neurointermediate lobe are α-melanocyte-stimulating hormone (α-MSH), corticotropin-like intermediate lobe peptide and β-endorphin (12). In the hypothalamus, POMC mRNA was also detected but at very low levels (9). In addition, a protein of the approximate size of POMC is synthesized in hypothalamic cultures and it has been shown to crossreact with ACTH and β-endorphin antibodies (13).

One can study the effect of glucocorticoids on *POMC* gene expression in intact animals by removing the tissue that produces these steroids, the adrenal cortex. When this is done, the ACTH level in the blood rises dramatically within a few minutes and then falls, only to rise again several hours later (14). In addition, Nakanishi *et al.* (15) have reported that POMC mRNA activity in total rat pituitary increases 2.5-fold after adrenalectomy. More recently, Schachter *et al.* (16) reported a 6.5-fold increase in anterior pituitary POMC mRNA levels 14 days after adrenalectomy. In this study, we have asked whether changes in blood levels of POMC peptides after adrenalectomy can be related to alterations in activity of the POMC gene(s) in the pituitary and hypothalamus as a result of adrenalectomy and glucocorticoid treatments.

We have measured gene expression by quantifying *POMC* gene transcription rates and the levels of POMC mRNA after adrenalectomy and subsequent glucocorticoid administration. To test for the possibility that *POMC* gene expression is regulated at the level of secretion, we have assayed the serum and anterior pituitaries from the same groups of animals for ACTH and β-endorphin peptide levels.

MATERIALS AND METHODS

Adrenalectomy and Dexamethasone Treatment. Six-week-old male Sprague–Dawley rats (University of Oregon strain derived from Charles River C/D strain) were used. Bilateral adrenalectomies and sham operations were carried out after administration of pentobarbital. During surgery, the sham-operated animals had their adrenals retracted and exposed but not excised. All animals were maintained on 0.9% saline after surgery. Dexamethasone (100 μg) or vehicle was injected into the pericardium in sterile saline. Animals subjected to long-term dexamethasone treatment were given twice daily subcutaneous injections of 80 μg. Animals were sacrificed by decapitation. The anterior lobe and neurointermediate/posterior lobe of the pituitary and hypothalami were dissected and immediately extracted or frozen in liquid nitrogen.

Corticosterone levels were determined by radioimmunoassay. Control and sham-operated rats had corticosterone at 20–50 pg/ml in their serum. In adrenalectomized rats, the corticosterone levels were below the level of detectability (5 pg/ml) in all but one individual.

Nucleic Acid Preparation. Total nucleic acid (TNA) was prepared from individual rat pituitary lobes. Fresh tissue or tissue flash-frozen in liquid nitrogen was homogenized in 1X SEH buffer (10 mM Hepes, pH 7.5/5 mM EDTA/1% $NaDodSO_4$) containing proteinase K (Boehringer) at 200 μg/ml in 0.4 ml (anterior lobe) or 0.25 ml (neurointermediate/posterior lobe).

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Abbreviations: ACTH, adrenocorticotropic hormone; POMC, proopiomelanocortin; MSH, melanocyte-stimulating hormone; TNA, total nucleic acid; CRF, corticotropin-releasing factor.

† Present address: Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

Homogenates were incubated for 15–30 min at room temperature and then extracted with 2 vol of phenol/chloroform, 1:1 (vol/vol) previously neutralized with Tris base. Ethanol (2.5 vol) was then added to the aqueous phase to precipitate the nucleic acid. A_{260} was used to determine the TNA concentration in 0.1X SEH buffer assuming that 20 A_{260} units = 1 mg/ml. TNA recoveries from the neurointermediate/posterior lobe varied from 6 to 10.5 μg per lobe and anterior lobe recoveries ranged from 75 to 125 μg each. Total RNA from the hypothalamus was prepared by the method of Chirgwin *et al.* (17). The hypothalami from five individuals were pooled for each group. Each group yielded approximately 150 μg of RNA.

POMC mRNA Determinations. The POMC mRNA levels in pituitary TNA extracts and hypothalamic RNA samples were determined by solution hybridization with ^{32}P-labeled single-stranded cDNA isolated from the POMC cDNA clone ME150 (18). The preparation of the single-stranded cDNA probe and the solution hybridization procedure were as described (9, 19) except that we used cytoplasmic RNA isolated from AtT20-D16v cells for which the POMC mRNA level had been previously determined as our hybridization standard.

***POMC* and Growth Hormone Transcription Rate Determinations.** Nuclei were prepared from the anterior and neurointermediate/posterior lobes of groups of eight animals at 1 and 4 hr after adrenalectomy and dexamethasone treatment and incubated with 300 μCi each of [α-^{32}P]UTP, -CTP, and -GTP (New England Nuclear) as described (20). Nuclear RNA was isolated (21) and hybridized to nitrocellulose filter discs (20) on which 2 μg of pBR322, pGH1 (22), an 800-base-pair rat growth hormone cDNA clone, or pMKSU16, a 923-base-pair mouse POMC cDNA clone (23), had been denatured and bound. After the hybridization, nonspecific radioactivity was removed by washing and RNase treatment (20) and the radioactivity on the filters was determined in a liquid scintillation counter. Specific transcription was calculated as the difference between radioactivity bound to control filters containing pBR322 and pMKSU16- or pGH1-containing filters in the same hybridization reaction. Results are expressed as cpm in the specific hybrid per 10^6 cpm in the hybridization mixture (ppm). Using RNA copies of plasmid clones (cRNA), we found hybridization efficiences of 35–45% (data not shown).

Of concern to us was the use of a mouse POMC cDNA clone to probe for the rat POMC transcript. Comparison of the pMKSU16 cDNA clone (23) with the corresponding sequence of the main exon of the rat *POMC* gene (24) indicates that there is >92% sequence homology between the two genes.

ACTH Determinations in Serum and Anterior Pituitaries. The ACTH antiserum used in the radioimmunoassays (14) is specific for the NH_2-terminal region of $ACTH_{1-39}$ as it crossreacts fully with $ACTH_{1-24}$. It does not crossreact with α-MSH, the COOH-terminal portion of ACTH ($ACTH_{24-39}$), β-endorphin, β-lipotropin, or β-MSH. The specificity of the β-endorphin antiserum is described in ref. 6. Acetic acid-soluble proteins were extracted from anterior pituitary lobes, lyophilized, suspended in buffer, and assayed for total protein and ACTH content (5).

RESULTS

When the adrenal cortex is removed, there is a rapid rise in plasma levels of ACTH followed by a fall and then another rise. Concomitant with the rise in plasma ACTH, there is a fall in the anterior pituitary level of the peptide followed by a gradual rise (14). In this study, we have asked to what extent these changes can be related to alterations in the activity of the *POMC* gene in the rat.

POMC mRNA Levels in the Anterior Pituitary After Adrenalectomy and Dexamethasone Treatment. The levels of POMC mRNA in the anterior pituitary were determined by cDNA solution hybridization analysis of TNA extracts at various times after adrenalectomy. After a lag of approximately 8 hr, the level of POMC mRNA showed a rapid increase with time (Fig. 1A), reaching 15 times that of the control level after 18 days. To determine whether the increase in POMC mRNA levels after adrenalectomy is due to removal of the endogenous source of glucocorticoids from the animals, some rats were treated with the synthetic glucocorticoid dexamethasone starting at day 8 after adrenalectomy. Animals were treated daily for up to 8 days and a time course of the POMC mRNA deinduction in the anterior pituitary was determined. As shown in Fig. 1*B*, by 8 hr after the initial dexamethasone administration, a decrease in POMC mRNA was observed. The results in Fig. 1 are in agree-

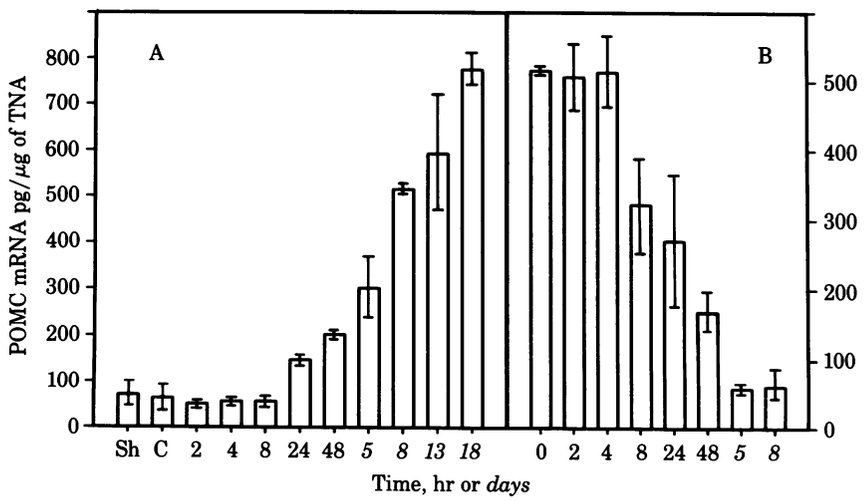

FIG. 1. Effect of adrenalectomy (*A*) and subsequent dexamethasone treatment (*B*) on POMC mRNA levels in the rat anterior pituitary. TNA from individual anterior pituitary lobes from young male rats was extracted and assayed for POMC mRNA content at various times after bilateral adrenalectomy and subsequent treatment with dexamethasone. Results represent mean ± SD of samples from three individuals. (*A*) TNA was prepared from pituitaries at various times after adrenalectomy and assayed for POMC mRNA content by cDNA solution hybridization. (*B*) Eight days after adrenalectomy, rats were treated with 160 μg of dexamethasone by injection into the pericardium and maintained on dexamethasone with twice daily subcutaneous injections of 80 μg. Sh, sham operated; C, control.

ment with a model of regulation in which glucocorticoids from the adrenal cortex play a role in repressing POMC mRNA levels in the anterior pituitary.

The POMC mRNA level in the anterior lobes of sham-operated animals was essentially the same as in unoperated (Fig. 1*A*) and 8-day dexamethasone-treated (Fig. 1*B*) animals. When unoperated animals were treated with dexamethasone for 8 days, the POMC mRNA levels were found to be 20% of control levels (data not shown). These results suggest that *POMC* gene expression in normal male rats is in a partially repressed state maintained by endogenous glucocorticoids.

Effect of Adrenalectomy on POMC mRNA in the Neurointermediate Lobe and Hypothalamus. *POMC* expression in the neurointermediate lobe is under negative regulation by dopaminergic compounds and γ-aminobutyric acid (11, 25). Even less is known about regulation of *POMC* in the hypothalamus.

To test the effects of glucocorticoids on *POMC* gene expression in the neurointermediate lobe, lobes were removed from unoperated rats, from rats that had been sacrificed 8 days after adrenalectomy, and from rats that had been sacrificed 16 days after adrenalectomy with dexamethasone (20 μg/ml) in their water for the last 8 days. TNA was extracted from individual neurointermediate lobes to determine POMC mRNA levels. As shown in Fig. 2, there were no major differences in POMC mRNA levels among the different groups of rats. A greater variation was detected in the POMC mRNA levels of neurointermediate lobes of animals within an experimental group than was generally observed in the anterior lobe (Fig. 2). This may be due in part to the removal of some anterior lobe tissue along with the neurointermediate/posterior lobe tissue during some of the dissections.

To test the effects of eliminating serum glucocorticoids on *POMC* gene expression in the hypothalamus, it was necessary to pool the tissue from five hypothalami per point to have enough RNA to measure POMC mRNA levels accurately. RNA was purified as described in ref. 1 from control animals and animals sacrificed 14 days after adrenalectomy and assayed for POMC mRNA levels (Fig. 2). The two hypothalamic RNA samples had almost identical levels of POMC mRNA, slightly >2 pg of POMC mRNA per μg of RNA each. This result indicates that glucocorticoids do not play a role in regulating POMC mRNA levels in the hypothalamus.

Transcription Rates of *POMC* and Growth Hormone After Adrenalectomy and Glucocorticoid Treatments. The pronounced changes in POMC mRNA levels in the anterior pituitary after adrenalectomy suggest that glucocorticoids may regulate *POMC* gene expression at the level of transcription. To test this possibility, we sought to assay *POMC* transcription rates by measuring the number of RNA polymerase molecules on *POMC* genes in isolated nuclei. In this experiment, animals were adrenalectomized 1 and 4 hr before sacrifice. Eight animals at each time were subjected to a sham operation. Sixteen animals at each time were adrenalectomized. Eight of the adrenalectomized animals were immediately injected with 50 μg of dexamethasone while the sham-operated and other eight adrenalectomized animals received equivalent volumes of vehicle.

The nuclei were purified and incubated with [α-^{32}P]NTPs. Nuclear RNA was then purified and hybridized to cDNA plasmid clones fixed to nitrocellulose filter discs. Transcription rates were simultaneously determined for *POMC* and growth hormone, a gene whose transcription rate has been shown to be stimulated by glucocorticoids in cell culture and *in vivo* (21).

The rate of *POMC* transcription increases by as much as 20-fold within 1 hr of adrenalectomy (Fig. 3*A*). The same elevated level of *POMC* transcription was observed 4 hr after adrenalectomy. The observed increase in *POMC* transcription was completely suppressed by treatment of the animals with dexamethasone immediately after surgery. The *POMC* transcription rate in the animals sacrificed 1 hr after sham operation is below the limits of detectability (0.2–0.5 ppm).

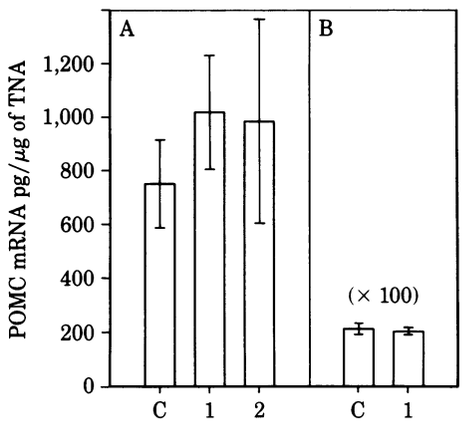

FIG. 2. Effect of adrenalectomy on POMC mRNA levels in the neurointermediate/posterior lobe and the hypothalamus. (*A*) Young male rats were adrenalectomized and TNA was prepared from individual neurointermediate/posterior lobes 8 days later (bar 1) or 16 days later, with dexamethasone at 20 μg/ml included in their water for the last 8 (bar 2). TNA samples were assayed for POMC mRNA levels by cDNA solution hybridization. Results represent mean ± SD for POMC mRNA levels from TNAs extracted from groups of three neurointermediate/posterior lobes. (*B*) Hypothalami from five individuals were pooled to obtain sufficient RNA to accurately measure POMC mRNA levels. Hypothalami were removed from five rats that had been adrenalectomized 14 days earlier (bar 1) and from five control rats (bar C). Total RNA was prepared from hypothalami by homogenization in guanidinium thiocyanate followed by centrifugation over a CsCl pad (17) and assayed for POMC mRNA content by cDNA solution hybridization.

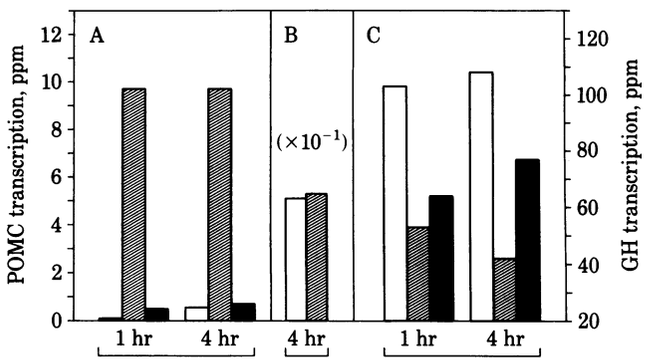

FIG. 3. Adrenalectomy causes elevation of *POMC* gene transcription in the anterior pituitary. Rats were subjected to bilateral adrenalectomy or sham operations. Half of the adrenalectomized animals were immediately injected with 100 μg of dexamethasone in the pericardium. At 1 and 4 hr after surgery, the animals were sacrificed, the pituitaries were removed, and the lobes were separated and frozen in liquid nitrogen. (*A* and *C*) Anterior lobe. (*B*) Neurointermediate lobe. Nuclei were purified (21) and assayed for *POMC* and growth hormone (GH) transcription rates by labeling nascent transcripts *in vitro* with [α-^{32}P]UTP, -GTP, and -CTP and hybridizing the radioactive RNA to plasmid DNA bound to nitrocellulose. All non-dexamethasone-treated animals were injected with an equal volume of saline vehicle. DNAs from pMKSU16 (23) and from pGH1 (22) bound to nitrocellulose filters were used as hybridization probes of specific *POMC* and *GH* transcription rates, respectively. pBR322 filters were included as controls and for background determinations. Results are reported in cpm of specific bound hybrid per 10^6 input cpm (ppm). □, Sham operated; ▨, adrenalectomized; ■, adrenalectomized, dexamethasone treated.

The assay of transcription rates 4 hr after adrenalectomy (Fig. 3*B*) showed that, in the neurointermediate lobe (*i*) transcription rate is approximately two orders of magnitude above that in the anterior lobe and (*ii*) there is no apparent effect on the *POMC* transcription rate under conditions that resulted in a marked increase in the anterior lobe after adrenalectomy.

The assay of the growth hormone transcription rate in the anterior lobe (Fig. 3*C*) showed that this rate is halved after adrenalectomy and is slightly increased after dexamethasone administration. Hence, the response of the growth hormone gene to adrenalectomy follows the predicted course and provides an independent check of the effectiveness of the surgery and the assay methodology. Although the growth hormone transcription rates do not vary over as large a range as the *POMC* rates, the assayed changes in growth hormone transcription are qualitatively what one would expect for a glucocorticoid-inducible gene *in vivo*.

Correlation of *POMC* Gene Expression with Pituitary and Serum ACTH Levels. An important aspect of this study was to correlate the temporal relationships of several parameters involved in the expression of a peptide hormone gene. Using *POMC* gene expression in the anterior pituitary as an example, we determined ACTH and β-endorphin levels in the plasma and anterior pituitary by radioimmunoassay and POMC mRNA levels by solution hybridization in animals subjected to adrenalectomy followed by dexamethasone treatment. The results are plotted in Fig. 4 as percentage of control values.

Within 2 hr after adrenalectomy, the plasma ACTH levels increased six-fold with a concomitant drop in pituitary ACTH (Fig. 4*A*). The levels of β-endorphin immunoreactivity changed in essentially a parallel fashion to ACTH (data not shown). This is consistent with a rapid increase in ACTH and endorphin release from the anterior pituitary following adrenalectomy, leading to a depletion of intracellular ACTH. By 24 hr after adrenalectomy, when the anterior pituitary POMC mRNA level had already increased >2-fold, a second increase in plasma ACTH was observed. There was then a more gradual rise in serum ACTH levels, roughly the same rate as POMC mRNA accumulation in the anterior pituitary. It was not until 5 days after adrenalectomy that the ACTH content in the anterior pituitary reached control levels. ACTH in the anterior pituitary then continued to increase during days 8–18 after adrenalectomy (data not shown) at about 1/3rd the rate of the POMC mRNA increase.

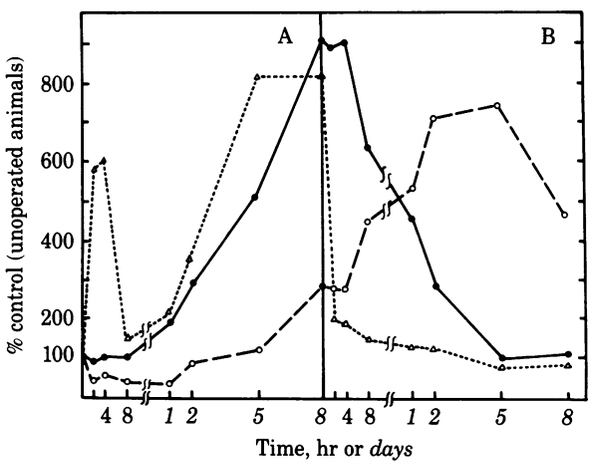

FIG. 4. Correlation of anterior pituitary POMC mRNA levels with plasma and pituitary ACTH levels after adrenalectomy (*A*) and subsequent dexamethasone treatment (*B*). Levels of ACTH in aliquots of serum (△) from groups of five animals were determined by radioimmunoassay (14). Acetic acid-soluble protein from the anterior pituitaries (○) of two or three individuals was extracted in the presence of protease inhibitors (5) and ACTH levels were determined by radioimmunoassay (5). POMC mRNA levels (●) are replotted from the data in Fig. 1. ACTH levels are plotted as the mean value for the individuals in each group. All results are plotted as percentage of levels determined for unoperated controls.

Within 2 hr after initiation of dexamethasone treatment, a sharp decrease in plasma ACTH levels was observed (Fig. 4*B*). Plasma ACTH then continued to decrease until day 5 of dexamethasone treatment and leveled off between days 5 and 8. Pituitary ACTH, in contrast, increased from the initiation of dexamethasone treatment to day 5 when it began to decrease. The increase in pituitary ACTH was likely due to rapid inhibition of ACTH secretion either directly by dexamethasone action on the pituitary or indirectly by inhibition of CRF secretion in the hypothalamus.

DISCUSSION

The data presented here support a model of *POMC* regulation in the rat anterior pituitary in which both *POMC* gene transcription and secretion of ACTH and endorphin-related peptides are in a partially repressed state in young male rats because of endogenous glucocorticoid levels in the blood. Bilateral adrenalectomy causes an increase in *POMC* gene transcription and ACTH secretion. The kinetics of the increase of ACTH in plasma indicates that glucocorticoids rapidly inhibit ACTH secretion *in vivo* (Fig. 4*B*). The rapid decrease in plasma ACTH after dexamethasone treatment subsequent to adrenalectomy supports this contention. The time course for the increase in the rate of ACTH secretion is clearly uncoupled from changes in POMC mRNA levels resulting in a depletion of intracellular ACTH from the anterior pituitary.

Because this experimental system involves the removal of a negative regulator, one must consider the decay time of serum glucocorticoids and the glucocorticoid–receptor complex in the cytoplasm and nucleus of target cells. Radioimmunoassay of serum corticosterone levels indicates that there was none detectable by 2 hr after adrenalectomy. An *in vivo* experiment in which [^{14}C]cortisol was injected into a rat indicates that the half-life of the steroid–receptor complex in hepatocyte nuclei is ≈0.5 hr (26), which is in agreement with values derived from *in vitro* studies using isolated hepatocyte nuclei (27). It is therefore extremely interesting that the elevated *POMC* gene transcription rate has already reached a maximal level by 1 hr after adrenalectomy (Fig. 3). While growth hormone gene transcription rates also change with a similar time course, although in the opposite direction, the magnitude of the response, both to adrenalectomy and to glucocorticoid treatment, is 1/10th as much [2-fold compared with 20-fold (Fig. 3)].

The data presented illustrate the high sensitivity of *POMC* gene expression in the anterior pituitary to changes in glucocorticoid levels, both at the level of transcription and at the level of ACTH secretion. It is possible that the *POMC* gene is the site of action of the glucocorticoid–receptor complex. This is based on the short time course of derepression of transcription of the *POMC* gene after adrenalectomy. A direct effect of glucocorticoids on *POMC* transcription in AtT20D16v cells after a 1-hr treatment has also been observed (unpublished results).

It is not possible to evaluate the role of positive regulators such as CRF on induction of POMC mRNA in these experiments. However, Swanson *et al.* (28) have recently reported a dramatic increase in the intensity of CRF-staining cell bodies in the paraventricular nucleus, suggesting increased CRF synthesis after adrenalectomy. Increased immunostaining for CRF in connecting fibers in the median eminence (the site of CRF release) is consistent with regulation of expression of *POMC* in

anterior pituitary cells by CRF. The increase in POMC mRNA levels in the anterior pituitary after intraventricular administration of CRF to male rats (unpublished data) also supports a role for CRF in *POMC* gene expression.

The rapid changes in ACTH serum levels after glucocorticoid treatment strongly suggest a separate and distinct action of glucocorticoids at the level of ACTH secretion, in agreement with results of Phillips and Tashjian (29), who report that short-term glucocorticoid treatment causes rapid inhibition of stimulated ACTH release in the AtT20-D16v cell line.

A rather surprising observation is the continued increase in POMC mRNA levels in the anterior pituitary through day 18 after adrenalectomy. There is no known precedent for so lengthy an increase in the level of a specific eukaryotic mRNA. By 18 days after adrenalectomy, the POMC mRNA level in the anterior lobe is only slightly below that of the neurointermediate lobe, a relatively pure population of corticotrophs (4).

Several possible explanations exist for this extended accumulation of POMC mRNA in the anterior pituitary. The corticotrophs in the anterior pituitary have been observed to increase both in size and number after adrenalectomy (30). Expression of the *POMC* gene in a cell type morphologically distinct from normal corticotrophs has been observed during postnatal development (31). Recruitment of another cell type in the anterior lobe (e.g., gonadotrophs) to begin expressing the *POMC* gene is therefore a possibility. Increases in size and number of cells expressing *POMC* in the anterior pituitary would contribute to the elevated level of *POMC* gene expression observed at longer times after adrenalectomy.

Within 5 days of dexamethasone treatment of adrenalectomized rats, the POMC mRNA levels in the anterior pituitary returned to control levels and did not decline any further (Fig. 1*B*). The half-time of the decline of POMC mRNA during dexamethasone treatment is approximately 24 hr (Fig. 1*B*). This serves as a rough estimate of the POMC mRNA half-life in the anterior pituitary. A half-life of 24 hr is considered typical for the stable class of eukaryotic mRNAs (32).

This study shows that *POMC* gene expression in the anterior pituitary is inhibited by serum glucocorticoids. Other studies indicate that CRF increases POMC mRNA levels in anterior pituitary cultures and that this effect is inhibited by glucocorticoids (unpublished data). Hence, CRF may be responsible for elevating POMC mRNA levels in the anterior pituitary after adrenalectomy.

Our studies show that glucocorticoids do not play a major role in the regulation of *POMC* gene expression in the neurointermediate lobe. However, there is evidence for dopaminergic inhibition of POMC mRNA levels (11) and for inhibition of γ-MSH secretion by γ-aminobutyric acid (25) in this lobe. Little is known about expression of *POMC* in the hypothalamus. Our data indicate that POMC mRNA levels are not regulated by glucocorticoids in the hypothalamus.

Regulation of *POMC* gene expression is best characterized in the anterior pituitary where regulation occurs at two levels of expression: transcription and secretion. The differences in regulation of *POMC* gene expression in the two lobes of the pituitary and hypothalamus make this system a valuable one for the study of neuropeptide gene expression.

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1. Herbert, E., Roberts, J. L., Phillips, M., Allen, R., Hinman, M., Budarf, M., Policastro, P. & Rosa, P. (1980) in *Frontiers in Neuroendocrinology*, eds. Martini, L. & Ganong, W. F. (Raven, New York), Vol. 6, pp. 67–100.
2. Vale, W., Speiss, J. & Rivier, J. (1981) *Science* **213**, 1394–1397.
3. Rosa, P. A., Policastro, P. & Herbert, E. (1980) *J. Exp. Biol.* **89**, 215–237.
4. Roberts, J. L., Chen, C.-L. C., Eberwine, J. H., Evinger, M. J. Q., Gee, C., Herbert, E. & Schachter, B. S. (1982) in *Recent Progress in Hormone Research*, ed. Greep, R. O. (Academic, New York), Vol. 38, pp. 227–256.
5. Roberts, J. L., Phillips, M., Rosa, P. A. & Herbert, E. (1978) *Biochemistry* **17**, 3609–3618.
6. Hinman, M. B. & Herbert, E. (1980) *Biochemistry* **19**, 5395–5402.
7. Yates, F. E. & Maran, J. W. (1974) in *Handbook of Physiology*, eds. Greep, R. O., Astwood, E. B., Knobil, E., Sawyer, W. H. & Geiger, S. R. (Waverly, Baltimore), Vol. 4, Part 2, pp. 367–404.
8. Rivier, C., Brownstein, M., Speiss, J., Rivier, J. & Vale, W. (1982) *Endocrinology* **110**, 272–278.
9. Civelli, O., Birnberg, N. C. & Herbert, E. (1982) *J. Biol. Chem.* **257**, 6783–6787.
10. Roberts, J. L., Chen, C.-L. C., Dionne, F. T. & Gee, C. (1982) *Trends NeuroSci.* **5**, 314–317.
11. Chen, C.-L. C., Dionne, F. & Roberts, J. L. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2211–2215.
12. Mains, R. E. & Eipper, B. A. (1979) *J. Biol. Chem.* **254**, 7885–7894.
13. Liotta, A. L., Gildersleeve, D. & Brownstein, M. J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1448–1452.
14. Kendall, J. W. (1971) in *Frontiers in Neuroendocrinology*, eds. Martini, L. & Ganong, W. F. (Oxford Press, London), pp. 177–201.
15. Nakanishi, S., Kita, S., Tau, S., Imura, H. & Numa, S. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3283–3286.
16. Schachter, B. S., Johnson, L. K., Baxter, J. D. & Roberts, J. L. (1982) *Endocrinology* **110**, 1442–1444.
17. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
18. Roberts, J. L., Seeburg, P. H., Shine, J., Herbert, E., Baxter, J. D. & Goodman, H. M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2153–2157.
19. Beach, L. R. & Palmiter, R. D. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2110–2114.
20. McKnight, G. S. & Palmiter, R. D. (1979) *J. Biol. Chem.* **254**, 9050–9058.
21. Evans, R. M., Birnberg, N. C. & Rosenfeld, M. G. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7659–7663.
22. Harpold, M. M., Dobner, P. R., Evans, R. M., Bancroft, F. C. & Darnell, J. E. (1978) *Nucleic Acids Res.* **5**, 2039–2053.
23. Uhler, M. & Herbert, E. (1983) *J. Biol. Chem.* **258**, 257–261.
24. Drouin, J. & Goodman, H. M. (1980) *Nature (London)* **288**, 610–613.
25. Tomiko, S. A., Taraskevich, P. S. & Douglass, W. W. (1983) *Nature (London)* **301**, 706–707.
26. Cake, M. H. & Litwack, G. (1975) in *Biochemical Actions of Hormones*, ed. Litwack, G. (Academic, New York), Vol. 3, pp. 317–389.
27. Baxter, J. D., Rousseau, G. G., Benson, C., Garcia, R. L., Ito, J. & Tomkins, G. M. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1892–1896.
28. Swanson, L. W., Sawchenko, P. E., Rivier, J. & Vale, W. W. (1983) *Neuroendocrinology* **36**, 165–186.
29. Phillips, M. A. & Tashjian, A. (1982) *Endocrinology* **110**, 892–900.
30. Childs, G. V., Ellison, D. G., Ramaley, J. A. & Unabia, G. (1982) *Endocrinology* **110**, 1676–1692.
31. Moriarty, C. M. & Moriarty, G. C. (1975) *Endocrinology* **96**, 1419–1425.
32. Puckett, L., Chambers, S. & Darnell, J. E. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 389–393.