Glucocorticoid Regulation of Pro-opiomelanocortin Gene Transcription in the Rat Pituitary*

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The effect of glucocorticoids on pro-opiomelanocortin (POMC) gene transcription in the rat pituitary has been investigated by an in vitro nuclear runoff transcription assay. Both dexamethasone and corticosterone had rapid inhibitory effects on POMC transcription in the anterior lobe but not in the intermediate lobe of the pituitary. These effects were maximal during the first hour after injection of the steroid. Bilateral adrenalectomy had a time-dependent stimulatory effect on anterior lobe POMC transcription, presumably because of the removal of endogenous glucocorticoids. This study shows that the well documented inhibitory effects of glucocorticoids on POMC mRNA in the pituitary anterior lobe are due at least in part to an inhibition in POMC mRNA synthesis.

Glucocorticoids play an important role in the regulation of the hypothalamic-pituitary-adrenal axis, the physiological system from which they are derived. The corticosteroids are secreted from the adrenal cortex primarily in response to ACTH. Secretion of ACTH is stimulated from the pituitary gland by a hypothalamic peptide, CRH. Glucocorticoids, in a classic endocrine feedback loop, produce inhibitory effects on secretion and decrease the content of CRH (1, 2) and ACTH (3, 4) in the hypothalamus and pituitary, respectively. It is the feedback effects of glucocorticoids on pituitary ACTH that we shall deal with in this study.

ACTH is synthesized together with β-endorphin and the melanocyte-stimulating hormones in the form of a precursor protein, POMC. Although POMC is synthesized in both the anterior and intermediate lobes of the pituitary, it is processed differently, with only the anterior lobe producing significant amounts of biologically active ACTH (for review, see Ref. 5). Interestingly, glucocorticoids elicit their negative effects only on the anterior lobe POMC system, not on the intermediate lobe POMC system (for review, see Ref. 6). Studies from many groups have shown that this negative regulation occurs at two levels: (a) inhibition of CRH-stimulated secretion of the mature peptides (2, 7–9) and (b) decreased POMC synthesis which results from a diminished level of cytoplasmic POMC mRNA (10–13). The glucocorticoid-mediated inhibition of CRH-stimulated POMC-derived peptide secretion requires 15–30 min to occur and appears to be indirect, requiring new protein synthesis (14). The glucocorticoid inhibition of POMC mRNA levels has a much longer time course of action, requiring at least 6 h for decreased levels to be observed (12, 15) and no new protein synthesis (6).

Although a great deal is known about the effects of glucocorticoids on POMC mRNA levels, little is known about mechanisms by which POMC mRNA levels change. There are many mechanisms by which these levels could be changed. Several genes have been shown to respond with increased levels of transcription upon exposure to glucocorticoids, e.g. growth hormone (16), MMTV (17), metallothionein (18), and tryptophan oxygenase (19). Conversely, proactin hnRNA levels decrease after addition of glucocorticoids to pituitary cell cultures (20). To determine whether or not glucocorticoids are acting in a similar fashion, i.e. having transcriptional effects on POMC gene expression, we have chosen to study the effect of glucocorticoids on POMC gene transcription in the anterior and intermediate lobes of the pituitary in an in vitro nuclear runoff transcription assay.

MATERIALS AND METHODS AND RESULTS

Glucocorticoid Effects on POMC Gene Transcription—Administration of dexamethasone to the rats for 4 days caused an approximate 2.5-fold decrease in the level of POMC gene transcription in the pituitaries of the dexamethasone-treated animal relative to non-steroid-treated animals (Fig. 1). This decrease could be due to a specific effect of the glucocorticoid on POMC transcription or to a change in the number of POMC-producing cells in the anterior lobe as a result of the glucocorticoid treatment. Since POMC-producing cells represent only 3 to 5% of the anterior lobe cells, a 2-fold change in the number of cells would not be readily observed in the total number of nuclei isolated. Indeed, there have been reports of glucocorticoid-mediated changes in POMC cell number in the anterior pituitary, but only after several days of adrenalectomy or steroid treatment. Thus, we analyzed short term effects (up to 6 h) of glucocorticoids on POMC gene transcription, time periods which do not affect the number of POMC-producing cells in either lobe of the pituitary.

The rate of POMC transcription was analyzed at various time periods up to 6 h after a single intraperitoneal injection of dexamethasone. The results of two such experiments are shown in Fig. 2. Variation was less than 10% between experi-

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1 The abbreviations used are: ACTH, adrenocorticotropic hormone; POMC, pro-opiomelanocortin; CRH, corticotropin-releasing hormone; MMTV, mouse mammary tumor virus.
Dexamethasone (100 μg) was injected intraperitoneally into animals and POMC transcription was measured with the nuclear runoff transcription assay in the anterior and neurointermediate lobe nuclei at varying time periods postinjection. The zero time point was from un.injected animals. Data are presented as the average of two separate experiments and the error bars represent the range of these determinations.

A different type of response is seen upon analyzing POMC gene transcription in the intermediate lobe (Fig. 2, bottom). Although there appears to be an inhibitory response, maximal inhibition is only 15% below normal values and does not occur until 1 to 2 h after administration of the glucocorticoid. In contrast to the anterior lobe, this slight inhibition in intermediate lobe POMC transcription returns to normal within 6 h.

The effect of the glucocorticoid treatment on cytoplasmic POMC mRNA levels was also measured. RNA was isolated from the postnuclear supernatant of each experimental group and POMC mRNA was quantitated by dot blot hybridization (data not shown). Dexamethasone had no detectable effect on POMC mRNA until 6 h after injection at which point there was a 30% decrease. There was no effect of the glucocorticoid injection on intermediate lobe POMC mRNA levels at any time point.

Dexamethasone has a higher affinity for the glucocorticoid receptor than the natural rat glucocorticoid, corticosterone (26). Further, dexamethasone does not bind to the natural glucocorticoid-binding globulin and is slowly cleared from the circulation of the rat (27). Thus, we performed a similar time course experiment using corticosterone, to ensure that the response observed in the transcription assay reflected a true glucocorticoid effect. A single intraperitoneal injection of corticosterone gave a different time course response relative to that of injected dexamethasone (Fig. 3). Maximal inhibition of anterior lobe POMC gene expression occurred between 30 and 45 min postinjection as compared to 15 min for dexamethasone. Corticosterone had a less potent inhibitory effect (3-fold) and POMC gene transcription returned toward normal levels more rapidly compared to the dexamethasone response. Corticosterone had no effect on intermediate lobe POMC transcription up to 6 h postinjection (data not shown).

In earlier experiments performed using the single steroid injection protocol, we often noticed variability in the level of POMC transcription at the 15-min postinjection time point. Usually POMC transcription was below control values, but occasionally it was elevated 10 to 20%. Because stressful stimuli cause release of POMC peptides from the pituitary (2, 3), we investigated the effect of injection of vehicle alone on POMC transcription. In two different experiments, shown in Fig. 4, the vehicle injection had a reproducible effect on POMC transcription in anterior lobe nuclei. At 15 min postinjection, there was an elevation of POMC transcription followed by a slight inhibition after 30 min. By 2 h postinjection, POMC transcription had returned to normal. Vehicle injection had no effect on POMC transcription in the neurointermediate lobe.

In order to analyze the effect of glucocorticoid deprivation on the transcription of the POMC gene, rats were adrenalectomized for varying time periods before being killed. Because of the stressful effects of ether anesthesia and surgery, POMC transcription was examined only after recovery from adrenalectomy. Adrenalectomy caused an increase in POMC gene transcription with a maximum of 5-fold stimulation after 1 week of adrenalectomy (Fig. 5). Longer time periods following adrenalectomy did not result in the same high level of POMC transcription, although the levels were still significantly above normal levels. In the intermediate lobe, in contrast, there is little response of POMC gene transcription to adrenalectomy. Maximal deviation from the control values is only 30%.

**DISCUSSION**

The *in vitro* nuclear runoff transcription assay measures the incorporation of radioactive RNA precursors into nascent POMC hnRNA chains which have been initiated *in vivo*. Thus, the observed decrease in radioactive POMC hnRNA bound to the nitrocellulose filters from glucocorticoid-treated animals would suggest that there are fewer RNA polymerase II complexes transcribing the POMC gene after treatment. The observation that the anterior lobe of the pituitary responds differently to glucocorticoids than does the intermediate lobe, both of which express the same POMC gene, shows that there are tissue-specific factors which modulate glucocorticoid actions. In this case, that factor is most likely the glucocorticoid receptor itself. Autoradiographic studies by Rees et al. (28) and Waremberg (29) showed that there are no translocatable glucocorticoid receptors in the intermediate lobe while the receptors are present in the anterior lobe POMC cells of normal and glucocorticoid-treated animals. Thus, the lack of a clear response of POMC transcription in the intermediate lobe to glucocorticoids is most likely because the POMC gene cannot "recognize" the steroid without the translocatable receptor.

The inhibition of POMC gene transcription in the anterior

![Graph showing time course of glucocorticoid effect on POMC transcription.](http://example.com/graph1.png)
lobe following glucocorticoid treatment appears to be a direct action of the glucocorticoid itself. The effect was observed with both a synthetic and the natural rat glucocorticoid. The stimulation of transcription following adrenalectomy with the resultant disappearance of plasma corticosterone also argues in favor of a specific glucocorticoid effect. The disappearance of these catecholamines with adrenalectomy probably does not play a significant role in the alteration of the anterior lobe POMC system because administration of glucocorticoids alone can restore POMC mRNA levels to normal (12, 13).

Adrenalectomy has complex effects on the anterior lobe POMC cells. There is a hypertrophy of the anterior lobe POMC cell to a volume three to five times its normal size that is maximal after 5 days (30, 31). This hypertrophy is accompanied by a proliferation of the rough endoplasmic reticulum and Golgi apparatus, the intracellular organelles involved in POMC synthesis. The POMC mRNA levels in the anterior pituitary are known to increase 5- to 10-fold in the same time period (13, 32). Thus, the increase in POMC transcription we observed following adrenalectomy was probably a reflection of both the increased level of POMC mRNA required to supply the approximate 10-fold increase in cytoplasmic volume devoted to POMC synthesis as well as to replenish POMC mRNA turning over in the larger volume. The decrease in POMC transcription between 1 and 2 weeks postadrenalectomy may be a result of the arrested increase in new POMC-producing cellular mass; the amount of transcription is still elevated relative to normal animals because the POMC cell nucleus must replenish POMC mRNA in a cytoplasm which is about five times larger with a corresponding increase in POMC mRNA levels.

The best evidence for a direct action of glucocorticoids on POMC transcription comes from the time course of the effect. Maximal inhibition was seen by 15 min postinjection, implying that inhibition of POMC transcription must have begun before that time. Glucocorticoids have a 20- to 30-min lag period before they can inhibit CRH-stimulated POMC secretion (2, 8). Indeed, using a corticosterone treatment identical with that in our studies, Buckingham (2) found that exposure of the animal to steroid for 15 min failed to inhibit ether-stimulated (i.e. CRH-mediated) POMC secretion. Thus, in these studies, at the time we observed maximal inhibition of POMC transcription, the anterior lobe POMC cell was still under CRH stimulatory control.

These studies show that, in the anterior pituitary, glucocorticoids are exerting a strong and rapid influence on the control of POMC gene transcription. Similar effects of glucocorticoids have been observed on the expression of MMTV (17), α2u-globulin (33), growth hormone (16) and metallothionein genes (34). In many of these examples, hybrid genes consisting of the five flanking promoter regions of these regulated genes have been fused to coding regions of nonregulated, nonregulated genes (35, 36). When transfected into cells which have glucocorticoid receptors, these hybrid genes are glucocorticoid responsive. This argues for the existence of particular sequences which specify the glucocorticoid responsiveness of those genes which are regulated. Utilizing a filter binding assay and monoclonal antibodies, Scheidereit et al. (37) have determined that a specific glucocorticoid receptor binding region in MMTV is 152 base pairs in length and of defined sequence. A similar sequence was found in metallothionein IIa (38) and in tryptophan oxygenase (39), both of which are glucocorticoid regulated. It remains to be shown whether or not a similar region exists in the POMC gene.

When rats are stressed, the 15-min time point will often be higher in the anterior lobes of the pituitary of steroid-injected animals than in those of control animals. This is always the case with vehicle-injected animals. This rapid increase may be a stress-mediated induction of POMC transcription, which conceivably acts via CRH stimulation of anterior lobe POMC secretion and synthesis. Whether CRH has a direct effect upon POMC mRNA accumulation or transcription has yet to be determined.

POMC transcription is only three to five times higher relative to total transcription in the neurointermediate lobe than in the anterior lobe, even though the POMC cells are about 20-fold higher percentage of the cell population (versus 80%). Since there is only one POMC gene in the rat haploid genome, this would suggest that the POMC gene is being transcribed at a different rate relative to total transcription in these two tissues, with the anterior lobe POMC gene being more active. It is already known that, on a per POMC cell basis, the level of cytoplasmic POMC mRNA is the same for these two tissues (13, 22, 32). Little is known about POMC mRNA turnover in the pituitary, so the significance of these observed transcription differences between lobes is not clear.

In calculating the number of RNA polymerase II molecules transcribing the POMC gene, one must make several assumptions: the rate of polymerase movement over the gene is constant, nascent transcripts are conserved, there are approximately 10,000 RNA polymerase II molecules per cell nucleus (40); and POMC cells compose about 4% of the anterior pituitary cell population. If these assumptions are valid, then there are approximately 1.6 RNA polymerases/POMC gene in the normal anterior lobe POMC cell. This is elevated to about 8 RNA polymerases (not taking into account any possible mitotic activity) in the adrenalectomized state and lowered to about 0.5 RNA polymerase in the dexamethasone-treated animal. It may be the case that the rate of polymerase movement over the POMC gene may vary by virtue of the presence of middle repetitive sequences within both of the POMC gene introns which may interact with other middle repetitive sequences or with RNA polymerase III to slow down RNA polymerase II movement.

Transcription of the POMC gene in the anterior lobe of the rat pituitary appears to be greatly influenced by the presence of glucocorticoids while the same is not true in the intermediate lobe. The fact that POMC transcription can be increased as well as decreased with respect to normal transcription suggests that POMC gene transcription is constantly in a state of suppression due to endogenous glucocorticoid action.

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REFERENCES
Glucocorticoid Regulation of POMC Gene Expression


SUPPLEMENTARY MATERIAL
Glucocorticoid Regulation of Pro-Orphanin-Family Gene Transcription in the Rat Pituitary

by James H. Hershwide and James L. Roberts

Methods and Materials

**Results and Discussion**: 

The results presented here were obtained using radioactive polyribonucleotides in studies with pituitary cells. These results indicate that glucocorticoids can modulate the expression of the POMC gene by influencing the transcriptional efficiency of this gene. The findings suggest that the glucocorticoid effect is not mediated by changes in protein synthesis but rather by post-transcriptional events. These results have important implications for understanding the mechanisms of glucocorticoid action in the pituitary gland and may provide insights into the pathophysiology of disorders associated with altered POMC gene expression.

Additional references are included to support the findings presented in this manuscript. Future studies will aim to further elucidate the molecular mechanisms underlying the glucocorticoid regulation of POMC gene expression.

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In order to assess the observed incorporation of radioactivity was due to RNA polymerase activity, the alphaventricular atria of POMC gene expression. At 100 ng/ml. A third set of control radiolabeled RNA was isolated from the treated sample. Incorporation of radiolabel was expressed as percentage of the total incorporation. The results indicated that the 24 hour period following the injection of dexamethasone significantly increased POMC gene expression. The overall 24 hour period following the injection of dexamethasone significantly increased POMC gene expression. Therefore, the optimal time for the dexamethasone treatment was determined, and the results showed that the dexamethasone treatment peaked at 24 hours post-injection. Additional, the dexamethasone treatment significantly increased POMC gene expression in the anterior pituitary. Therefore, the optimal time for the dexamethasone treatment was determined, and the results showed that the dexamethasone treatment peaked at 24 hours post-injection. Additional, the dexamethasone treatment significantly increased POMC gene expression in the anterior pituitary.

Table 1: Analysis of POMC Gene Expression

<table>
<thead>
<tr>
<th>Treatment</th>
<th>POMC mRNA Level (CPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>120 ± 10</td>
</tr>
<tr>
<td>Dexamethasone 1h</td>
<td>240 ± 20</td>
</tr>
<tr>
<td>Dexamethasone 24h</td>
<td>480 ± 30</td>
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</tbody>
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**Figure 3:** Dexamethasone treatment significantly increased POMC gene expression in the anterior pituitary. The treatment was administered 1 hour and 24 hours post-injection. The results showed that the 24 hour treatment peaked at 24 hours post-injection. Additional, the 24 hour treatment significantly increased POMC gene expression in the anterior pituitary.

**Figure 4:** Long term dexamethasone treatment caused an increase in anterior pituitary POMC gene expression. The treatment was administered 1 hour and 24 hours post-injection. The results showed that the 24 hour treatment peaked at 24 hours post-injection. Additional, the 24 hour treatment significantly increased POMC gene expression in the anterior pituitary.
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