Mechanism of Glucocorticoid-induced Increase in Pancreatic Amylase Gene Transcription*

Craig D. Logsdon, Karen J. Perot, and Alex R. McDonald

From the Cell Biology Laboratory and the Department of Medicine, Mount Zion Hospital and Medical Center and the Department of Physiology, University of California, San Francisco, San Francisco, California 94120

To determine the mechanism(s) responsible for glucocorticoid-induced increases in amylase content in pancreatic acinar AR42J cells, we examined the effects of dexamethasone on amylase protein biosynthesis, steady-state mRNA levels, and gene transcription. Dexamethasone treatment led to a dose-dependent increase in amylase synthesis which was one-half maximal at 2 nM and maximal at 100 nM where a 6-fold increase was achieved. This dexamethasone-induced increase in amylase synthesis was detectable after 12 h, one-half maximal after 19 h, and approached maximal after 72 h. Dexamethasone treatment also increased amylase mRNA levels in a time- and dose-dependent manner in parallel with the changes in amylase synthesis. Nuclear RNA transcript elongation (run-on) assays indicated that amylase gene transcription was also increased in a time- and dose-dependent manner. Glucocorticoid enhancement of amylase gene transcription occurred relatively slowly, with a 6-fold increase occurring after 48 h of treatment with 100 nM dexamethasone. Thus, the effects of glucocorticoids on pancreatic amylase gene transcription fully accounted for the increased levels of amylase mRNA, synthesis, and content. However, due to the slow time course of dexamethasone induction of amylase gene expression we evaluated the possibility of glucocorticoid induction of a regulatory protein. We found that inclusion of cycloheximide or puromycin during dexamethasone treatment blocked the induction of amylase mRNA. These data suggest that the glucocorticoid-induced increase in amylase gene transcription requires induction of an unidentified regulatory protein(s).

Glucocorticoids are important regulators of pancreatic amylase (AMY-2) gene expression in vivo. Glucocorticoids induce a precocious initiation of amylase synthesis in fetal pancreas of rats and chicks (1-3), and in newborn animals, injection of glucocorticoids leads to a premature increase in amylase content to the level seen in adults (4, 5). In the adult rat, adrenalectomy reduces pancreatic amylase concentration (5-7). Furthermore, adrenalectomy selectively decreased amylase mRNA levels, and these effects were reversed by treatment with exogenous glucocorticoids (7). However, since these studies were conducted in vivo, they are difficult to interpret due to potential complex indirect hormonal interactions. Therefore, to understand the direct interaction of glucocorticoids with the exocrine pancreas, we have utilized a cultured line of rat pancreatic acinar cells, the AR42J cells (8). In AR42J cells, we have previously shown that glucocorticoids increase amylase concentration and translatable mRNA levels in vitro. In addition, in AR42J cells glucocorticoids have pleiotropic effects on cellular differentiation, leading to increases in the numbers of secretory organelles (8) and secretogogue receptors (9).

The mechanisms, however, whereby glucocorticoids exert their effects on pancreatic amylase gene expression are unknown. In most cases, glucocorticoid regulation of specific mRNAs is a result of enhanced gene transcription (for review see Ref. 10). However, steroids have also been reported to affect message stabilization (11), processing (12), and nuclear transport (13). In addition, the effects of glucocorticoids may be primary or secondary. A primary response is characterized by a rapid increase in the transcription of the inducible gene and by insensitivity of the mRNA induction to protein synthesis inhibition. Examples of primary responses include glucocorticoid induction of mouse mammary tumor virus (14) and metallothionein (15) genes. Secondary responses to glucocorticoids require protein synthesis for transcription of the inducible gene and typically show a lag period between administration of the hormone and initiation of the response. Glucocorticoid-induced increases in transcription of tryptophan 2,3-dioxygenase (16) and α₂-globin (17) appear to be secondary responses.

In the current study, the molecular mechanisms whereby glucocorticoids regulate pancreatic amylase gene expression in AR42J cells have been investigated in detail. We have correlated the time and the dose dependence of the dexamethasone effects on amylase protein: biosynthesis, steady-state mRNA levels, and gene transcription. In addition, the effects of inhibiting protein synthesis on the ability of dexamethasone to increase amylase gene expression were investigated. The results are consistent with an effect of glucocorticoids on amylase gene transcription which requires the induction of one or more pancreatic regulatory genes.

EXPERIMENTAL PROCEDURES AND RESULTS

Effects of Dexamethasone on Amylase Synthesis in AR42J Cells—We have previously shown that incubation of AR42J

1 Portions of this paper (including "Experimental Procedures, part of "Results," Fig. 2, and Footnote 2) are presented in miniprint at the end of this paper. The abbreviations used are: SDS, sodium dodecyl sulfite; kb, kilobase pair; TCA, trichloroacetic acid; DTT, dithiothreitol; TES, N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 87M-1513, cite the authors, and include a check or money order for $3.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Glucocorticoids Increase Amylase Transcription

In the presence of dexamethasone leads to increased cellular content and synthesis of amylase (8). In the current study, to determine the time course of the increase in amylase synthesis, AR42J cells were incubated in the presence of 100 nM dexamethasone for various lengths of time and then pulsed with [35S]methionine, and they newly synthesized proteins were separated by polyacrylamide gel electrophoresis (Fig. 1). Dexamethasone led to an increase in amylase synthesis which was first detected at 12 h, one-half maximal at 19 h, and a maximal 6-fold increase was observed by 48 h (Fig. 2a).

To determine the time course of the increase in amylase synthesis, cells were treated with various concentrations of dexamethasone for 48 h and then pulsed with [35S]methionine and the newly synthesized proteins analyzed. Dexamethasone increased amylase synthesis one-half maximally at ±2 nM and maximally at 100 nM (Fig. 2b).

Effects of Dexamethasone on Amylase mRNA Concentration in AR42J Cells—Increased amylase synthesis could be due to either increased translation of amylase mRNA or to increased levels of amylase mRNA. We previously reported that pretreatment of AR42J cells with dexamethasone lead to an increase in translatable mRNA for amylase (8). In the current study, dexamethasone effects on amylase mRNA content were analyzed using a cDNA probe for amylase.

To determine the time course of the increase in amylase mRNA, AR42J cells were incubated in the presence of 100 nM dexamethasone for various lengths of time and analyzed for their amylase mRNA content by slot-blot hybridization. Amylase mRNA concentration increased significantly after 12 h, one-half maximally after 18 h, and maximally after 48 h of treatment, where a maximal 6-fold increase was achieved (Fig. 2a). This time course closely paralleled that of the effects of dexamethasone on amylase synthesis. To determine the selectivity of the effects of dexamethasone for amylase gene expression, the concentrations of chymotrypsin and ribonuclease mRNA were also analyzed (Fig. 3). The mRNA levels for these other digestive enzymes were unaffected by treatment with dexamethasone.

To analyze the dose dependence of the increase in amylase mRNA levels, AR42J cells were treated with various concentrations of dexamethasone for 48 h and then analyzed. Dexamethasone increased amylase mRNA levels one-half maximally at 2 nM and maximally at 100 nM (Fig. 2b).

Effects of Dexamethasone on Amylase Gene Transcription—Increases in amylase mRNA could be due to changes in amylase gene transcription, amylase mRNA stability, or amylase mRNA stability. Little reduction in amylase mRNA content was seen after 8 h of actinomycin treatment of cells either treated with or without dexamethasone (data not shown) indicating that amylase mRNA is extremely stable. To study transcription directly, transcription rates were assessed by nuclear run-on. Nuclei isolated from AR42J cells treated with dexamethasone for various amounts of time were allowed to elongate previously initiated transcripts in the presence of [32P]UTP (Fig. 4). Dexamethasone increased transcription of the amylase gene significantly after 6 h, one-half maximally after 14 h and maximally after 48 h of treatment where a 6-fold increase was achieved (Fig. 2a). This time course of dexamethasone increase in transcription was relatively slow, but clearly preceded the increases observed in steady-state mRNA levels and amylase biosynthesis. The elongation of amylase mRNA was markedly inhibited by α-amanitin, indicating that amylase transcription occurred via RNA polymerase II (Fig. 4). To determine the selectivity of the effects of dexamethasone for amylase gene transcription, the effects on ribonuclease gene transcription were also analyzed (Fig. 4). The transcription rate for ribonuclease was not significantly affected by treatment with dexamethasone.

The dose dependence of the dexamethasone-induced increase in amylase gene transcription was also investigated.
Glucocorticoids Increase Amylase Transcription

### DISCUSSION

Regulatory effects of hormones on pancreatic digestive enzyme biosynthesis have been demonstrated to occur both at the level of mRNA concentration (7, 8, 25) and mRNA translation (26, 27). The current report is the first direct demonstration of transcriptional regulation of pancreatic digestive enzyme gene expression. We have shown that glucocorticoids regulate pancreatic amylase gene transcription in the AR42J cell line, and this increase in amylase gene transcription fully accounts for the observed increase in amylase mRNA, synthesis, and content. Furthermore, the effects of glucocorticoids are selective for amylase, as other digestive enzyme mRNA levels were not increased by dexamethasone treatment of AR42J cells.

Amylase gene expression in vivo is increased by administration of glucocorticoids (1-5) or by administration of insulin to diabetic animals (25, 28) and decreased by the administration of cholecystokinin (29). Although interactions between glucocorticoids and other hormones, particularly insulin, may be important, glucocorticoids have clear effects in the presence of the negligible insulin concentrations which exist in serum-containing medium and in the absence of insulin in serum-free medium.3

Several lines of evidence suggest that the effect of glucocorticoids on amylase gene transcription in AR42J cells requires the induction of an unknown regulatory protein(s). First, the kinetics of dexamethasone induction of amylase gene transcription are slow. Significant effects were not observed before 6 h of treatment, and maximal effects were not observed before 24-48 h. Second, inhibition of protein synthesis blocked the ability of dexamethasone to increase amylase gene expression. It is unlikely that the reduction of dexamethasone-induced amylase gene expression by protein synthesis inhibitors was due to a nonspecific effect. Both cycloheximide and puromycin, agents with different mechanisms of action, blocked the effects of dexamethasone.

The function of this glucocorticoid-induced regulatory protein(s) is unknown. One possibility is that the glucocorticoid-responsive protein interacts with the amylase gene to increase transcription. Thus, glucocorticoids may not interact directly with the amylase gene, and the response may be a true secondary interaction. A glucocorticoid response element consensus sequence exists upstream of the promoters for AMY-1 and AMY-2 genes (30). However, the presence of a consensus sequence is not proof of function. For example, in the rat α1-acid glycoprotein gene, two sites having a precise or near match with the glucocorticoid response element consensus hexamer were found to be inactive in mediating dexamethasone regulation of a reporter gene when transfected into L cells (31). Another possibility is that the protein factor induced by glucocorticoids may act in concert with the glucocorticoid receptor to activate amylase gene transcription. In this latter case the glucocorticoid response element may be utilized, but require the presence of another glucocorticoid-responsive protein to increase the activity of the amylase promoter. This type of interaction has been reported for glucocorticoid regulation of the α1-acid glycoprotein gene (32).

Whether the regulatory protein(s) responsible for the effects of glucocorticoids on amylase gene transcription are also responsible for the wide variety of glucocorticoid effects on AR42J cell differentiation is unknown. These differentiation effects include inhibition of cell growth (8), increases in intracellular organelles involved in the synthesis and secretion of digestive enzymes (8), and increases in the numbers of cholecystokinin receptors (9). Glucocorticoids have previously been suggested to increase the expression of a regulatory factor required for activating the entire set of differentiation-dependent genes in an adipogenic cell line (33). Recently, glucocorticoids have been shown to increase amylase gene expression in a mouse hepatoma cell line (30). That glucocorticoids also had global effects on the differentiation of these hepatoma cells was suggested by the long time course required and the fact that after dexamethasone treatment, the hepatoma cells expressed the pancreas-specific AMY-2 gene, which is normally expressed only in pancreatic acinar cells.

It will be important to determine the nucleotide sequences involved in this response to glucocorticoids. Osborn et al. (34) have recently reported the expression of an amylase minigene in transgenic mice. The minigene, which included 208 base pairs of 5'-flanking sequence and 300 base pairs of 3'-flanking sequence, was expressed at high levels in a tissue-specific manner and was regulated by glucocorticoids. This approach should help identify the cis-acting regions of the amylase gene. However, in the transgenic animal it is difficult to

---

3 C. D. Logsdon, K. J. Perot, and A. R. McDonald, unpublished observations.

### Table I

**Effects of cycloheximide and puromycin on dexamethasone-induced increases in amylase mRNA levels**

<table>
<thead>
<tr>
<th>Additions</th>
<th>Amylase mRNA (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone (100 nM)</td>
<td>262 ± 6 (n = 5)</td>
</tr>
<tr>
<td>Dexamethasone (100 nM) plus cycloheximide (10 μM)</td>
<td>124 ± 3 (n = 5)</td>
</tr>
<tr>
<td>Dexamethasone (100 nM) plus puromycin (3 μM)</td>
<td>128 ± 14% (n = 3)</td>
</tr>
<tr>
<td>Cycloheximide (10 μM)</td>
<td>101 ± 4 (n = 5)</td>
</tr>
<tr>
<td>Furomycin (3 μM)</td>
<td>99 ± 3 (n = 3)</td>
</tr>
</tbody>
</table>

Dexamethasone increased amylase gene transcription one-half maximally at 2 nM and maximally at 100 nM (Fig. 26).

**Cycloheximide Inhibits the Dexamethasone-induced Increase in Amylase mRNA**—Since the effects of dexamethasone on amylase gene transcription required several hours of hormone treatment, we examined whether this effect might require the synthesis of regulatory protein(s). AR42J cells were treated for 12 h with 100 nM dexamethasone in the presence or absence of 10 μM cycloheximide at which point amylase mRNA was determined. This concentration of cycloheximide was found to inhibit 90 ± 1% (n = 4) of AR42J cell protein synthesis. Cycloheximide treatment of control cells lead to a small decrease in the amylase mRNA content (Table I). In contrast, cycloheximide blocked the dexamethasone-stimulated increase in amylase mRNA content.

To test whether this effect was due to an inhibition of protein synthesis by cycloheximide, and not to some nonspecific effect, similar experiments were conducted with puromycin, another protein synthesis inhibitor which acts via different cellular mechanisms. Puromycin at a concentration of 3 μM inhibited protein synthesis by 82 ± 7% (n = 3) and also inhibited the dexamethasone-induced increase in amylase mRNA (Table I). At these concentrations, neither cycloheximide nor puromycin had significant effect on cell viability as determined by trypan blue exclusion, and the inhibition of protein synthesis was completely reversible (data not shown).
ascertain whether regulation is direct or indirect and primary or secondary. Thus, identification of trans-acting factors may require the use of AR42J cells in vitro.

Acknowledgments—We wish to thank John A. Williams and Ira Goldfine for critical reading of the manuscript, M. E. Hodes for use of the human amylase cDNA, Graeme Bell for the rat chymotrypsin cDNA, Raymond MacDonald for the rat ribonuclease cDNA, and Lily Ruslin for valuable technical assistance.

REFERENCES


Supplemental Material to
Heterogeneity of Glucocorticoid Induced Increase in Pancreatic Amylase Gene Transcription
by
Craig D. Logsdon, Karen J. Pericot, and Alex R. McDonald

EXPERIMENTAL PROCEDURES

Cell Culture

AR42J cells were maintained as subconfluent cultures in Dulbecco’s modified Eagle’s medium containing penicillin, streptomycin, amphotericin B and 10% fetal bovine serum. Cells were incubated at 37°C in a humidified atmosphere of 5% CO2 in air and 100% relative humidity.

RNA isolation and quantitation

RNA was isolated from AR42J cells by a modification of the method of Chirgwin et al. (18). AR42J cells (2 x 10^7) were suspended in Tris-Cl (pH 7.4, 1.0 M NaCl, 0.05 M sodium citrate), homogenized (sonication), and extracted with phenol/chloroform/isoamyl alcohol (50:48:2) and precipitated with ethanol at -80°C. RNA was washed twice in 4 x SSC with 0.1% SDS at 55°C for 30 minutes, then in 2 x SSC with 0.1% SDS at 37°C for 30 minutes, in 1 x SSC with 0.1% SDS at 55°C for 30 minutes, and finally in 0.1 x SSC with 0.1% SDS at 80°C for 30 minutes.

Hybridization

Hybridizations were carried out with the following probes: pCXP33, isolated from an AR42J cDNA library and identified as possessing a 4.9 kb amylase cDNA insert by cotransfection mapping and hybridization with a human pancreatic amylase cDNA; pCSIP7, containing a 0.35 kb rat chymotrypsin B (rCB) or pCSIP10, containing a 4.6 kb cDNA for rat chymotrypsinogen (rC) cDNAs were labeled with [32P]dCTP (New England Nuclear) using a NEN nick translation kit (Bethesda Research Laboratories, Bethesda, MD). The specific activity of the probes was routinely 5 x 10^8 cpm/ug. Filters were prehybridized at 47°C for 4 hours in 5 ml of solution containing 5 x SSC, deionized 50% formamide, 1 x Denhardt's reagent (0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 10 ng phosphate buffer, 7.0, 0.01 M Na2HPO4, 0.14 M sodium cacodylate, 100 ul nonradioactive salmon sperm DNA, and 50 ug/ml poly(C). Hybridizations were performed at 42°C overnight with gentle mixing in 2 ml of the above solution containing 5 x SSC, deionized 50% formamide, 1 x Denhardt's reagent (0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 10 ng phosphate buffer, 7.0, 0.01 M Na2HPO4, 0.14 M sodium cacodylate, 10 ng/ml nonradioactive salmon sperm DNA, and 50 ug/ml poly(C). Filters were dried and exposed to X-ray film. Radioactivity determined by scanning the autoradiograph with a densitometer equipped with an area integrator (Shaffer Scientific Instruments, San Francisco, CA). Densitometric analysis of the autoradiographs was performed using a computerized densitometer (Laboratory Imaging, Denver, CO). The density of each sample was calculated for the linear portion of the curve by linear regression, and the relative intensity of each sample calculated at a common fold dilution.

Nuclear Transcription Assay

AR42J cells were incubated in the absence and presence of 100 nM dexamethasone for various times up to 24 hours. After incubation, 1 x 10^6 cells were washed twice in phosphate buffered saline. nuclei were prepared by resuspending cells in 10 ml of lysing buffer (0.5 M sucrose, 1 M NaHepes, pH 8.0, 10 mM MgCl2, 0.5 uM EDTA and 0.1% Triton X-100) and homogenized with
Nuclear for hybridization buffer (10 pelleted at minutes at 10,000 extracted and ethanol precipitated. Each a-amanitin. Nascent RNA chains were elongated in set of nuclei run-on assay method described by Nelson and Groudine (23).

10 strokes of a tight-fitting pestle of a Dounce homogenizer. The nuclei were centrifuged and rinsed 4 times in lysis buffer before layering over a sucrose cushion, consisting of the above buffer in 1 M sucrose, and pelleted at 800 g for 3 minutes at 4°C. The purified nuclei were resuspended to a final concentration of 2 × 10⁶/ml in storage buffer (150 mM Hepes pH 8.0, 0.5% glycerol, 9 mM MgCl₂, 0.1 mM EDTA) and used immediately or stored at -70°C.

Transcriptional analysis of the amylose gene was carried out by the nuclear run-on assay method described by Nelson and Groudine (23). One set of nuclei (5 × 10⁷) for each condition were treated with 2 ug/ml a-amanitin. Nascent RNA chains were elongated sequentially with RNase-free DNase, protease K, phenol-chloroform extracted and ethanol precipitated. Each sample was next centrifuged for 5 minutes at 10,000 g in a microfuge and the pellet was suspended in hybridization buffer (10 µM TES [pH 7.4], 0.2% npo, 10 mM EDTA, 0.3 M NaCl), in 40% glycerol, and used immediately or stored at -70°C for various times. Autoradiographs were prepared with X-ray film at -70°C for 24 h. After developing, autoradiographs were scanned with a densitometer.

For comparisons between nuclei, equal numbers of counts per minute of run-on products typically around 10⁶ cpm/ml were hybridized to duplicate nitrocellulose filters containing 5 ug of plasmids for either amylose, ribonuclease, or pBR322. To prepare the nitrocellulose filters, the plasmids were linearized and denatured in 0.3 M NaOH for 1 hour at 65°C, neutralized in 1 M ammonium acetate pH 7.0, and transferred to nitrocellulose using a slot blot apparatus. Nitrocellulose filters were prehybridized in the above hybridization solution for 2 hours at 65°C and then hybridized to the run-on products in hybridization solution for 48 hours at 65°C. After hybridization, the filters were washed for 2 hours at 65°C in 2 x SSC, then incubated for 30 minutes at 37°C in 2 x SSC with 100 ug/ml RNase A. Further washing was then carried out for 30 minutes at 65°C in 1 x SSC plus 0.1% SDS, 1 hour in 0.1 x SSC, and then washed twice with 100% ethanol and air dried. Autoradiographs were scanned with a densitometer. Image analysis was performed by the method of Laemmli in 10% polyacrylamide slab gels (12.5 cm x 12.5 cm x 1.5 mm). After electrophoresis, the gels were stained with 0.05% Coomassie blue in 10% trichloracetic acid for 30 min and destained in 7 M methanol (vol/vol) and 7% acetic acid (vol/vol) dried under vacuum and heat and autoradiographed with Kodak X-ray film for 24 h at -70°C. After developing, autoradiographs were scanned with a densitometer.

Protein Synthesis

Protein synthesis was determined by measurement of [3H]leucine incorporation into trichloracetic acid precipitable material. AR42J cells were plated into 35 mm culture plates for 24 h and treated with or without 10 µM cycloheximide or 3 µg/ml puromycin for 30 min before the addition of 100 nM dexamethasone. Cells were cultured for a further 12 h before the addition of [3H]leucine (50 µCi/ml). Incorporation was terminated 30 min later by removing the medium and replacing it with 1 ml of saline 154 mM NaCl at 4°C containing 1% nonidet P-40. AR42J cells were then washed twice with 1 ml of saline at 4°C, scraped into 1 ml of water and sonicated with a probe-type sonicator. Aliquots were removed for protein determination, and samples were precipitated with trichloracetic acid (TCA) final concentration, 1% at 4°C. The precipitates were washed twice with additional cold 10% TCA and dissolved in 0.5 ml of 0.1 N HCl. Radioactivity was measured by liquid scintillation counting. Incorporation of [3H]leucine was calculated as nanocuries per milligram cell protein and expressed as percent control.

Determination of amylose synthesis was determined by pulse-labeling with 1µCi [3H]leucine as reported previously (8). The amylose band, identified by molecular weight and by immunoprecipitation with an anti-amylose antibody, was previously found to be the most prominent labeled band (8). Therefore, in the current study amylose was identified by molecular weight and by prominence. Polyclonal antibodies against amylose (PAN) of equal numbers of trichloracetic acid precipitable counts per minute (from each cell lysate in the presence of 1.19 RNase was performed by the method of Laemmli in 10% polyacrylamide slab gels (12.5 cm x 12.5 cm x 1.5 mm). After electrophoresis, the gels were stained with 0.05% Coomassie blue in 10% trichloracetic acid for 30 min and destained in TCA methanol (vol/vol) and 74% acetic acid (vol/vol) dried under vacuum and heat and autoradiographed with Kodak X-ray film for 24 h at -70°C. After developing, autoradiographs were scanned with a densitometer.

**RESULTS**

![Figure 1](image.png)

**Figure 1.** Time-course and dose-dependence of dexamethasone effects on amylose synthesis, RNA levels, and gene transcription. AR42J cells were pretreated either with 100 nM dexamethasone for various lengths of time or with various concentrations of dexamethasone for 48 h. The cells were then analyzed as to the level of amylose synthesis (obscure, amylose level), the level of RNA levels (obscure), and the level of gene transcription (obscure) in the presence of dexamethasone effects. Results are expressed as a percent of control and are means ± SEM for 3-4 experiments. Dose-dependence of dexamethasone effects. Results are expressed as a percent of maximal and are means ± SEM for 3-4 experiments.