Transcriptional Regulation of Glutamine Synthetase Gene Expression by Dexamethasone in L6 Muscle Cells*

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Dexamethasone increases glutamine synthetase activity and mRNA abundance in L6 muscle cells in culture, apparently by a glucocorticoid receptor-mediated process. The data in this report reveal that increased glutamine synthetase mRNA abundance is attributable at least in part to an enhanced rate of transcription of the glutamine synthetase gene. "Nuclear runoff" assays of glutamine synthetase gene expression were performed with purified myonuclei from dexamethasone-treated or untreated L6 skeletal muscle cells. These assays showed glutamine synthetase transcription to be increased approximately 2-fold as early as 1 h after incubation of cells with dexamethasone (10⁻⁷ M); there was no increase in the rate of transcription of the β-tubulin gene, which served as a control. The increase in glutamine synthetase gene transcription correlates with increased glutamine synthetase enzymatic activity after dexamethasone treatment. Studies with actinomycin D indicated that half-life of glutamine synthetase mRNA (7-8 h) is not altered by dexamethasone. Therefore, the degradation of glutamine synthetase mRNA is not affected by dexamethasone, and the increased glutamine synthetase mRNA level is attributable to increased transcription. The dexamethasone-mediated increase in glutamine synthetase mRNA abundance is glucocorticoid receptor-mediated; RU38486 (a glucocorticoid receptor blocker) completely blocked the effect of dexamethasone. The dexamethasone-mediated increase in glutamine synthetase gene transcription and steady-state mRNA level was not blocked by cycloheximide, indicating a direct effect.

Skeletal muscle produces and releases glutamine, which serves as a substrate for the energy metabolism of a number of cells, tissues, and organs, including intestine, lymphocytes, kidney, and, possibly, brain (Ardawi and Newsholme, 1985; Goldstein, 1986; Tildon and Zielke, 1988). In fact, skeletal muscle serves as the principal reservoir of body glutamine (Goldstein, 1986). The synthesis and release of glutamine by muscle into the blood stream are increased under stressful conditions such as fasting and trauma (Goldstein, 1986; Rennie et al., 1986). However, the quantity of glutamine contained in muscle proteins is insufficient to account for all the glutamine released by muscle (Chang and Goldberg, 1978; Komini et al., 1954). Therefore, glutamine is synthesized de novo in muscle (Goldberg and Chang, 1978; Odessey et al., 1974); glutamine synthetase appears to catalyze the final step (Ruderman and Berger, 1974) in this synthesis.

Muscle glutamine synthetase gene expression is increased in experimental diabetes (Feng et al., 1990a) and following denervation (Feng et al., 1990b). It is also increased after administration of dexamethasone to rats in vivo (Max et al., 1988) and to L6 muscle cells in culture (Max et al., 1987). The dexamethasone-dependent increase in glutamine synthetase activity is thought to be mediated by interaction of the steroid with muscle glucocorticoid receptors (Max et al., 1987). Dexamethasone causes a significant increase in the steady-state level of glutamine synthetase mRNA (Max et al., 1987). Increased mRNA level could be attributable either to increased transcription or to increased mRNA stability. In the present study, we have investigated these possibilities. The data to be presented show that dexamethasone increases the rate of transcription of the glutamine synthetase gene via a glucocorticoid receptor-mediated mechanism without influencing the half-life of glutamine synthetase mRNA.

EXPERIMENTAL PROCEDURES

Materials

L6 cells, originally isolated by Yaffe (1965), were provided by Drs. T. H. Oh and G. J. Markelos (Dept. of Anatomy, University of Maryland School of Medicine, Baltimore, MD). L-[U-¹⁴C]Glutamic acid (specific activity, 100-150 mCi/mmol) was purchased from Research Products International (Mount Prospect, IN). Tissue culture plasticware was purchased from Corning Glass Works (Corning, NY). Culture media and sera were products of Gibco. All other chemicals, unless otherwise noted, were obtained from Sigma.

Cell Culture

L6 myotubes were maintained in culture and processed for enzyme assay as described by Max et al. (1987) and Konagaya et al. (1988).

Enzyme Assay

Glutamine synthetase activity was assayed using the method of Rowe (1985) as described by Smith et al. (1984), except that the glutamate concentration was 5 mM. These assays separate product from substrate by ion exchange chromatography. The substrate is [1-¹⁴C]Glutamate.

Protein Assay

The protein concentration was determined according to the method of Lowry et al. (1951), using crystalline bovine serum albumin as the standard.

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mRNA Analysis

Total cellular RNA was isolated from L6 muscle cells using the guanidine isothiocyanate procedure (Chirgwin et al., 1979). Equal amounts of total cellular RNA were fractioned by agarose electrophoresis in formaldehyde (Rave et al., 1979). The RNA was then transferred to nitrocellulose filters; the filters were baked in vacuo at 80 °C for 2 h and hybridized with a radioactively labeled glutamine synthetase cDNA probe (Thomas, 1980). The glutamine synthetase probe was isolated from a rat brain Agt11 cDNA library by complementary screening with a hamster genomic clone of glutamine synthetase (Max et al., 1988; Meawar et al., 1988). The clone was plaque purified and subcloned into the EcoRI site of the vector pBluescript II+. The 2.4-kb rat glutamine synthetase cDNA insert contains both translated and 3′-untranslated regions. The blots also were hybridized to a rat brain cyclophilin cDNA probe as an internal control gene (Lad et al., 1990; Koletsky et al., 1986). Both glutamine synthetase and cyclophilin probes were labeled to high specific activity through the “random primed” labeling method of Feinberg and Vogelstein (1983, 1984).

In Vitro Transcription Assay

Isolation of Nuclei from L6 Cells—The medium was aspirated and the cells washed twice with cold phosphate-buffered saline (Ca2+- and Mg2+-free). The cells were harvested quickly into cold phosphate-buffered saline by scraping. The cells were pelleted, rinsed gently with phosphate-buffered saline, and repelleted. The pellet was resuspended in cold cell lysis buffer (10 mM Tris, pH 8.4, 1.5 mM MgCl2, 70 mM NaCl), Nonidet P-40 was added to a final concentration of 0.25%, and the contents of the tube were mixed by vortexing. The suspension was incubated on crushed ice for 15 min and checked for cell lysis by light microscopy. Nuclei were isolated from the cell lysis solution by centrifugation at 100,000 × g on a discontinuous sucrose gradient. The nuclear pellet was washed with buffer (70 mM NaCl, 20 mM Tris, pH 8.0, 1.0 mM MnCl2, 5 mM MgCl2, 20% glycerol, and 14 mM β-mercaptoethanol). The nuclei were repelleted and diluted in portions containing 106 nuclei in 500 μl of nuclear freezing buffer (40% glycerol, 10 mM MgCl2, 250 mM KCl, 50 mM Tris, pH 7.8, and 10 mM creatine phosphate). The nuclei were frozen at −160 °C in liquid nitrogen until used. Nuclei prepared in this fashion were transcriptionally competent for up to 6 months.

In Vitro Transcription Reaction—Nuclear transcription activity was determined by measurement of the incorporation of [3P]-labeled GTP (Amersham Corp.) into RNA transcripts elongated in vitro using a modification of the protocol of McKnight and Palmiter (1979). The nuclei were thawed slowly on ice and vortexed. An equal volume of 2 × reaction buffer was added to 106 nuclei (2 × reaction buffer contains 2 mM dithiothreitol; ATP, CTP, and UTP (2 mM each); 2 μl (10 μg/ml) of creatine kinase; 6 μl of RNasin (Promega Biotec); 250 μl of [3P]GTP (3000 Ci/μmol); and 5 μM unlabeled GTP). The reactions were mixed well and incubated at 29 °C for 45 min. 50 μl of RQ DNase I (Promega Biotec) were added to stop transcript elongation. Proteinase K digestion was performed twice, followed by RNA extraction with phenol and chloroform. After final RNA precipitation, incorporated radioactivity was counted by trichloroacetic acid precipitation. Specific RNA transcripts were detected by hybridization of the 3P-labeled RNA (2 × 106 cpm) to the corresponding cDNA on Nytran filters. 500 ng of denatured glutamine synthetase probe were immobilized on the filter. The β-tubulin probe used as a control in Northern and “runoff” assays contains most of the coding sequence for the human β-tubulin message, transcribed from the ubiquitously expressed M40 β-tubulin gene, and all of the 3′-untranslated region (Hall et al., 1983). Equal amounts of labeled total RNA were used in hybridizations, which were carried out at 42 °C for 22 h following 24-h prehybridization with 50% formamide at 42 °C. mRNA was quantified by densitometry.

mRNA Stability Measurements—mRNA stability was measured by actinomycin D chase (Cote and Gagel, 1987; Lindenbaum et al., 1988). Cells were incubated with control medium or with medium containing dexamethasone for 24 h, after which actinomycin D (4 μg/ml) was added to control and treated dishes. Total RNA was isolated at the time of actinomycin D addition or at different times thereafter. Northern blot analysis was performed to determine mRNA levels.

RESULTS

In vivo administration to rats caused an apparently greater increase in the glutamine synthetase mRNA than enzyme activity in muscle (Max et al., 1988). Time and dose-response studies were conducted to ascertain the correspondence of enzyme activity and mRNA level in cell culture. Dexamethasone (10−7 M), caused a striking increase in glutamine synthetase specific activity (Fig. 1). The increase was greater than 8-fold after 48 h of incubation of L6 myotubes with dexamethasone. Northern analysis (Fig. 2) showed that glutamine synthetase mRNA abundance was increased by approximately 2-fold as early as 2 h after incubation with dexamethasone (10−7 M) and eventually attained a relative level that was comparable to the increase in enzyme activity (Figs. 1 and 2). Dose-response experiments (Fig. 3) showed that the effect of dexamethasone was maximal at 10−7 M (cf. Max et al., 1987). This increase was accompanied by a corresponding increase in the glutamine synthetase mRNA level (Fig. 4, A and B).

We (Max et al., 1988) and others (Bhandari et al., 1986; Kumar et al., 1986) reported that glutamine synthetase mRNA exists as two species of 2.8 and 1.4 kb. As seen in Figs.
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**Fig. 3.** Dose response of effect of dexamethasone on glutamine synthetase activity in L6 muscle cells in culture. Cells were exposed to dexamethasone for 24 h. Data are means ± S.D., n = 3. *Significantly different from control, p < 0.05. Experimental procedures are described in the text.

**Fig. 4.** Dose response of effect of dexamethasone on glutamine synthetase mRNA abundance in L6 muscle cells in culture. Cells were exposed to dexamethasone for 24 h. Duplicate lanes are shown for each dexamethasone dose. A, Northern blot. B, densitometric scan of Northern blot (2.8-kb species only). Experimental procedures are described in the text. Data are means ± S.D., n = 2.

2 and 4, both species are regulated to the same extent by dexamethasone in L6 cells. The existence of these different-sized glutamine synthetase mRNAs has been attributed to selective use of two different polyadenylation signals present in the last exon (Kuo and Darnell, 1989). Dexamethasone caused no changes in the steady state levels of β-tubulin mRNA in L6 cells (data not shown).

To determine the possible mechanism(s) underlying the increase in glutamine synthetase mRNA abundance, nuclear transcript elongation (“runoff”) assays were performed using purified L6 myonuclei. As seen in Fig. 5 (A and B), the transcription rate more than doubled by 3 h after incubating L6 cells with 10^{-7} M dexamethasone; transcription activity increased to about 4 times control by 8 h and remained at this level for up to 12 h of incubation with dexamethasone (Fig. 4B). There was no change in transcription of the β-tubulin gene (Fig. 5B). Further, no signal was detectable when the “runoff” assay was carried out using the Bluescript plasmid (Fig. 5B). Transcription of the glutamine synthetase gene was blocked completely by α-amanitin (1 μg/ml; data not shown).

It is possible that the increase in mRNA level could reflect a decreased rate of mRNA degradation. This possibility appears to be excluded by the experiment depicted in Fig. 6, in which the half-life of glutamine synthetase mRNA, as determined by actinomycin D chase, is seen to be unaltered by dexamethasone. The t_{1/2} (estimated from Fig. 6) of glutamine synthetase mRNA is 7-8 h in the presence or absence of dexamethasone. Sakar and Chaudhury (1983) estimated the t_{1/2} of glutamine synthetase mRNA to be 6-8 h. Thus, it appears that increased glutamine synthetase gene transcription underlies increased glutamine synthetase activity and mRNA level in dexamethasone-treated L6 cells.

In our earlier report, we showed that the dexamethasone-induced increase in glutamine synthetase activity was blocked by RU38486, a glucocorticoid antagonist (Max et al., 1987), thus supporting the notion that glutamine synthetase is responsive to glucocorticoid receptor activation. In Fig. 7, we show that the dexamethasone-mediated increase in the glutamine synthetase mRNA level similarly is blocked by RU38486, further demonstrating that the effect of dexameth-
asone is mediated via interaction of the steroid with intracellular glucocorticoid receptors, which exist in L6 cells (Konagaya et al., 1988). RU38486 alone had no effect on glutamine synthetase mRNA levels. Finally, Fig. 7 also shows that the dexamethasone-mediated increase in glutamine synthetase mRNA level is not blocked by 10 μM cycloheximide, indicating that the dexamethasone effect is direct and does not require additional protein synthesis. Cycloheximide at this dose inhibited [3H]leucine incorporation into proteins in L6 cells by 95% (data not shown).

**DISCUSSION**

Glutamine may exert direct control over the rate of muscle protein turnover (Rennie et al., 1986). Therefore glutamine synthesis via glutamine synthetase and release via the glutamine carrier (Rennie et al., 1986) appear to be implicated in the control of muscle mass. Thus, understanding the control of glutamine production in muscle is of paramount physiological importance. Because glutamine synthetase catalyzes the final step in glutamine synthesis in muscle (Ruderman and Berger, 1974), it is critical to understand control of the expression of this enzyme. In an earlier report (Max et al., 1987), we showed that glucocorticoids, including dexamethasone, increase glutamine synthetase activity and mRNA abundance in L6 muscle cells in culture. In this report, we have conducted careful time and dose-response studies to ascertain the correspondence of increases in enzyme activity and mRNA level. These data are in contrast to our results with rat skeletal muscle *in vivo* (Max et al., 1988). In intact skeletal muscle, the dexamethasone-mediated increase in mRNA level was greater than the increase in enzyme activity. In L6 cells dexamethasone caused similar increases in enzyme activity and mRNA level (Figs. 1–4). Thus, controls of enzyme activity additional to those seen in cell culture may be exerted in intact muscles. As we showed for enzyme activity in cell culture (Max et al., 1987), increased glutamine synthetase mRNA abundance following dexamethasone treatment of L6 cells was blocked by a glucocorticoid antagonist, RU38486 (Konagaya et al., 1986). Thus, the effect of dexamethasone on muscle weight (Konagaya et al., 1986), glutamine synthetase activity (Max et al., 1987, 1988), and glutamine synthetase mRNA level (Max et al., 1988) require interaction of the glucocorticoid with intracellular receptors, which exist in intact muscle (Konagaya et al., 1987) and in L6 cells (Konagaya et al., 1988). Such receptor-mediated effects suggest that glutamine synthetase gene transcription is enhanced via a receptor-mediated mechanism. We now have shown that this is the case: dexamethasone increases the rate of transcription of the glutamine synthetase gene in L6 muscle cells (Fig. 5). It is not known whether the glutamine synthetase gene has classic glucocorticoid regulatory elements either in the 5' region or within introns. Increased glutamine synthetase mRNA level may be attributable solely to enhanced transcription, because dexamethasone caused no change in the half-life of glutamine synthetase mRNA (Fig. 6). However, alterations by dexamethasone of glutamine synthetase mRNA processing or nucleocytoplasmic transport cannot be ruled out. Finally, the cycloheximide experiment (Fig. 7) suggests the effect of dexamethasone may not require additional protein synthesis to increase the transcription of the glutamine synthetase gene.

Glutamine synthetase is the first protein shown to be induced by glucocorticoids in muscle at the mRNA level. The present demonstration that the dexamethasone-mediated increase in glutamine synthetase mRNA level is attributable to increased transcription adds to our understanding of the action of glucocorticoids on muscle and may lead to understanding of mechanisms by which glucocorticoids cause muscle atrophy and the possible role of glutamine in that process.

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**REFERENCES**

Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) *Biochemistry* 18, 5294–5300

**FIG. 7.** Effect of RU38486 (10^{-3}M) or cycloheximide (10 μM) on dexamethasone-mediated increase in glutamine synthetase mRNA level in L6 muscle cells in culture. Cells were pretreated with RU38486 for 1 h and then exposed to dexamethasone for 24 h. Cells were exposed to dexamethasone and cycloheximide simultaneously for 24 h. A, Northern blot. B, densitometric scan of Northern blot. Experimental procedures are described in the text. dex, dexamethasone; Ru, RU38486; cyh, cycloheximide.
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Transcriptional regulation of glutamine synthetase gene expression by dexamethasone in L6 muscle cells.

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