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DIFFERENTIAL INDUCTION OF TRANSCRIPTION FOR GLUCOCORTICOID-RESPONSIVE GENES IN CULTURED RAT HEPATOCYTES

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SUMMARY: Dexamethasone rapidly stimulated transcription of the tyrosine aminotransferase and metallothionein-I genes -- but not of the phosphoenolpyruvate carboxykinase gene -- in rat hepatocytes cultured in serum-free medium. This differential response was not observed for cyclic AMP. The results suggest that the phosphoenolpyruvate carboxykinase gene -- but not the tyrosine aminotransferase and metallothionein-I genes -- requires a factor which is permissive for stimulation of transcription by the glucocorticoid receptor.

Regulation of gene transcription by glucocorticoids involves interaction of the hormone-receptor complex with a glucocorticoid response element(s) (GRE) , usually located upstream of the transcription start site of the gene. Although initially derived from studies with the long terminal repeat of the mouse mammary tumor virus, this model of glucocorticoid action has been supported by subsequent studies of a variety of cellular genes (reviewed in 1,2). However, a number of observations suggest that the hormone-receptor complex may be necessary but not sufficient for hormone-dependent stimulation of transcription of several genes expressed in liver; e.g., albumin (3) and tryptophan oxygenase (4,5). These genes apparently require factors in addition to the glucocorticoid receptor which are permissive for hormonal induction of transcription.

In cultured rat hepatocytes, dexamethasone induction of mRNAs encoding PEPCK and several of the urea cycle enzymes occurred only after a lag period of about 4 h (6,7). In addition this induction was blocked by an inhibitor of protein synthesis (6,7). We set out to determine whether the lag in induction was a general feature of glucocorticoid - responsive genes in rat hepatocytes cultured in serum-free medium or whether distinct subclasses of glucocorticoid-responsive genes could be identified, particularly with regard to differences in

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Abbreviations used: TAT, tyrosine aminotransferase; MT-I, metallothionein-I; PEPCK, phosphoenolpyruvate carboxykinase (GTP); GRE, glucocorticoid response element.

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induction of transcription shortly after exposure to dexamethasone. In the present experiments we analyzed the glucocorticoid-dependent induction of genes which have been shown previously to contain functional GREs. GREs have been identified for the TAT (8) and PEPCK (9,10) genes of rat and for the human MT-IIA gene (11). A DNA fragment encompassing 600 base pairs of 5' flanking region of the rat MT-I gene contains a DNA sequence homologous to the consensus GRE (12) and also mediates glucocorticoid induction of a reporter gene in transfection experiments (DD, unpublished results). Our results suggest that a factor(s) distinct from the glucocorticoid receptor is required for induction of PEPCK gene transcription by dexamethasone but this putative factor is not required for induction of TAT and MT-I gene transcription.

MATERIALS AND METHODS

Procedures for isolation and culture of rat hepatocytes, isolation and quantification of mRNAs, and transcription run-on assays have been described (7,13,14). Cloned cDNAs encode rat PEPCK (15), mouse TAT (16), mouse MT-I (17), and rat β-tubulin (18).

RESULTS AND DISCUSSION

Differential induction of mRNAs. In contrast to our previous observation of a 4-h lag for PEPCK mRNA induction in cultured hepatocytes (6), TAT and MT-I mRNAs were induced by dexamethasone with no apparent lag in five independent experiments (Figure 1). We noted previously that the kinetics of PEPCK mRNA induction suggested that initiation of induction required synthesis of some intermediate gene product (6). This notion was supported by the observation that the induction was blocked by cycloheximide (6). Conversely, the rapid induction of TAT and MT-I mRNAs implied that ongoing protein synthesis was not required for initiation of these responses. However, analysis of the induction of TAT and MT-I mRNAs by dexamethasone in the presence of cycloheximide was inconclusive since cycloheximide itself proved to be a potent inducer of these two mRNAs in cultured hepatocytes (VLN, DD, and SMM; unpublished results). Induction of TAT and MT-I mRNAs by cycloheximide alone has been observed also by other investigators (19,20). Whether the correlation between a rapid response to dexamethasone and inducibility by cycloheximide signifies an important aspect of the mechanism of induction by dexamethasone or is merely fortuitous is not known.

Differential induction of transcription. The different kinetics of mRNA induction suggested that a differential response of the three genes might be observed also at the level of transcription. In particular, the kinetics of mRNA induction (Fig. 1b) suggested that substantial increases in TAT and MT-I gene transcription rates should be readily apparent by 1 h after addition of dexamethasone. The striking result of the transcription assays, in each of two independent experiments, was that dexamethasone strongly induced transcription

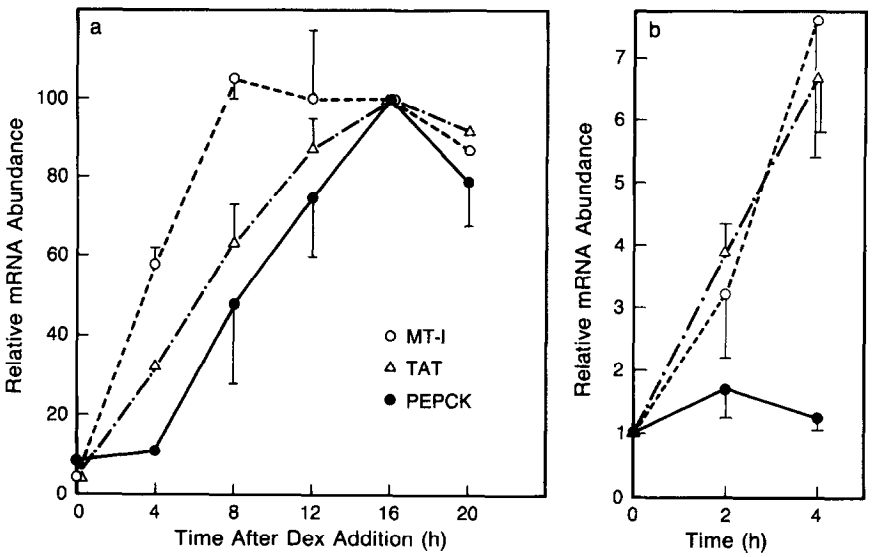

Figure 1. **Time course of mRNA induction by dexamethasone in cultured rat hepatocytes.** Hepatocytes were isolated from male Sprague-Dawley rats and cultured in serum-free medium (6,7). Dexamethasone (1μM) was added to cultured hepatocytes 28 h after plating, and total RNA was extracted at the times indicated. Relative mRNA abundance was determined by quantitative Northern blot analysis. Results for all three mRNAs were obtained from analyses of a common set of five independent hepatocyte preparations. (a) Messenger RNA abundance was normalized to the 16 h value within each experiment. Results are expressed as the average and range for two independent hepatocyte preparations, with the exception that values for TAT and MT-I mRNAs at 20 h are for a single preparation. Range bars are not shown when exceeded by the dimensions of the symbol. (b) Messenger RNA abundance was normalized to the control value within each experiment. Values represent means $\pm$ S.E.M. for three separate experiments. Data for PEPCK mRNA are reproduced by permission from Biochemical Journal, vol. 246, pp. 237-240, copyright 1987, The Biochemical Society, London.

of the TAT and MT-I genes within 1 h but there was no significant change in transcription of the PEPCK gene (Figure 2). In contrast, PEPCK gene transcription was clearly induced by a cyclic AMP analog within 1 h (Figure 2), thus demonstrating that the failure to induce PEPCK transcription was specific for dexamethasone and not due to a general lack of inducibility of the PEPCK gene. Specificity of hormonal induction in these experiments was demonstrated by the absence of any change in β-tubulin gene transcription and by the different patterns of transcriptional response elicited by dexamethasone and the cyclic AMP analog.

In multiple independent experiments TAT and MT-I mRNA abundance and transcription rates increased shortly after exposure to dexamethasone, while PEPCK mRNA abundance and transcription rate remained unchanged at early times (Fig. 1 and 2). Since the induction of TAT and MT-I gene transcription demonstrated the presence of a functional glucocorticoid receptor in the cultured hepatocytes, the failure to induce PEPCK gene transcription could mean that this gene does not contain a functional GRE and thus is

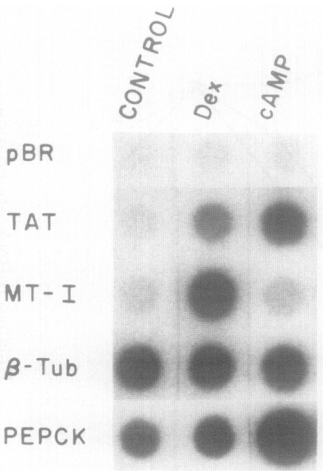

Figure 2. **Induction of gene transcription by dexamethasone and CPT-cyclic AMP in cultured rat hepatocytes.** At 28 h after plating, cultured hepatocytes were exposed to 1 μM dexamethasone or 50 μM 8-(4-chlorophenylthio)-cyclic AMP for 1 h. Nuclei were isolated and transcription run-on assays were performed as described (13,14). Equal amounts of labelled RNA were added to each hybridization reaction. After hybridization and washing, results were visualized by exposing the filters to X-ray film at -70° C in the presence of intensifying screens. These results from a single hepatocyte preparation are representative of two independent sets of experiments. Abbreviations: β-Tub, β-tubulin; pBR, pBR322; Dex, dexamethasone; cAMP, 8-(4 chlorophenylthio) cyclic AMP.

induced as a secondary response to dexamethasone. However, dexamethasone stimulates PEPCK gene transcription six-fold within 30 min in a rat hepatoma cell line (21), a finding which is not characteristic of a secondary response. Moreover, functional GREs within the PEPCK promoter have been identified by transfection of PEPCK promoter-reporter gene fusions into hepatoma cells (9,10). We therefore favor the hypothesis that the PEPCK gene -- and perhaps several other genes with similar response kinetics in cultured hepatocytes (5,7) -- requires a factor which is permissive for stimulation of transcription by the glucocorticoid receptor-hormone complex. However, this putative factor is not required for induction of the TAT and MT-I genes. This factor apparently is present in the H4IIEC3 rat hepatoma cell line but is either absent or inactive in rat hepatocytes cultured in hormone- or serum-free medium. A similar proposal was derived from studies on regulation of tryptophan oxygenase gene transcription (5).This hypothesis also predicts that the 5' flanking region of the PEPCK gene contains two classes of DNA sequences required for glucocorticoid response: one which contains a binding site for the glucocorticoid receptor and another which presumably contains a binding site for the putative permissive factor. Consistent with this hypothesis, mutations in DNA sequences outside the GRE have been found to reduce or abolish glucocorticoid induction of the tryptophan oxygenase gene (4) and the mouse mammary tumor virus (22-24). In addition, transfection studies using DNA constructs containing a single GRE plus a binding site for an additional transcription factor have demonstrated that any one of several

transcription factors can potentially modulate glucocorticoid responsiveness in vivo (25). Alternatively, induction of PEPCK gene transcription could involve association of the putative factor with the glucocorticoid receptor but not directly with the DNA, in which case mutational analysis of the PEPCK promoter would identify only binding sites for the glucocorticoid receptor.

Results of the present study differ in two significant respects from earlier observations (3,5) on dexamethasone induction of specific gene transcription in cultured rat hepatocytes: First, the lack of rapid transcriptional induction was observed for some but not all glucocorticoid-responsive genes. Second, lack of rapid transcriptional induction was specific for glucocorticoids and was not observed for cyclic AMP. Our results, as well as those of other investigators (3,5), indicate that culture of rat hepatocytes under certain conditions may allow identification of requirements for specific factors involved in the glucocorticoid response of specific genes. More generally, these results suggest a possible mechanism for specifically modulating the responses of subsets of glucocorticoid-regulated genes within different cell types and during development.

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