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Transcriptional and Posttranscriptional Mechanisms of Glucocorticoid-Mediated Repression of Phosphoenolpyruvate Carboxykinase Gene Expression in Adipocytes

Sylvie Franckhauser-Vogel,[1] Jocelyne Antras-Ferry,[2] Danielle Robin,[1] Pierre Robin,[1] and Claude Forest[1]*

[1]Centre de Recherche sur l'Endocrinologie Moléculaire et le Développement, Meudon, France
[2]Faculté de Pharmacie, Laboratoire de Biologie Cellulaire et Végétale, INSERM U49, Rennes Cedex, France

Abstract Glucocorticoids exert pleiotropic effects, among which negative regulation of transcription has been recognized as of crucial importance. While glucocorticoids induce phosphoenolpyruvate carboxykinase (PEPCK) gene expression in liver cells, it represses gene activity in adipose cells. We used the 3T3-F442A adipocytes to analyze the underlying mechanisms. In these cells, the synthetic glucocorticoid dexamethasone exerts a dominant repression either on basal or on β-agonist stimulation of PEPCK gene expression. To determine whether glucocorticoid action required protein synthesis, we employed cycloheximide, anisomycin, and puromycin, three different translation inhibitors. None of these affected induction by isoprenaline or repression by dexamethasone of isoprenaline stimulation. In contrast, dexamethasone inhibitory action on basal PEPCK mRNA was totally prevented by the three translation inhibitors. Time courses of glucocorticoid action on basal and on induction by β-agonist were similar. Half-maximal effect of dexamethasone on isoprenaline-induced PEPCK mRNA was obtained at about 10 nM, a tenfold higher concentration than that observed for the reduction of basal mRNA. Using the transcription inhibitor DRB, we showed that dexamethasone did not alter mRNA half-life, while isoprenaline strongly stabilized mRNA. In a 3T3-F442A stable transfectant bearing −2,100 base pairs of the PEPCK promoter fused to the chloramphenicol acetyltransferase (CAT) gene, isoprenaline stimulated CAT activity, whereas dexamethasone reduced basal and isoprenaline-induced CAT expression. Hence, β-agonists exert both transcriptional and posttranscriptional regulation, while glucocorticoid action is purely transcriptional. However, mechanisms of glucocorticoid repression of basal and of β-agonist stimulation appear different. J. Cell. Biochem. 66:386–393, 1997.

Key words: glucocorticoids; PEPCK; gene expression; adipocytes; dexamethasone

Cytosolic phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) (PEPCK) catalyzes the conversion of oxaloacetate to phosphoenolpyruvate. This step is critical for adipose glyceroneogenesis and for hepatic gluconeogenesis [Rongstad, 1979; Reshef et al., 1969, 1970]. PEPCK activity and mRNA are elevated in liver and in adipose tissue when the supply of glucose is limited (e.g., during starvation). Under this circumstance, PEPCK activity is required for hepatic glucose production and for synthesis of glycerol-3-phosphate from pyruvate, lactate, or amino acids in adipocytes. Glycerol-3-phosphate is necessary for esterification of fatty acids into triacylglycerol. Starvation is correlated to elevated concentrations of cyclic AMP–inducing hormones (glucagon, epinephrine, etc.), which are strong and rapid inducers of PEPCK gene expression in liver and in adipose tissue both in vivo [Hopgood et al., 1973; Meyuhas et al., 1976] and in cultured cells [Lamers et al., 1982; Franckhauser et al., 1995]. In hepatic cells, glucocorticoids are stimulatory [Lamers et al., 1982; Sasaki et al., 1984; Nebes and Morris, 1987]. In contrast, in adipocytes, glucocorticoids repress PEPCK gene expression and inhibit stimulation by cAMP [Franck-

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*Correspondence to: C. Forest, Centre de Recherche sur l'Endocrinologie Moléculaire et le Développement, CNRS, 9 rue Jules Hetzel, 92190 Meudon, France.

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hauser et al., 1995]. This opposite regulation of the PEPCK gene by glucocorticoids is intriguing at first glance. It is, however, physiologically relevant. Glucocorticoids are stress hormones, and stress is a situation during which energy must be available to tissues like muscle. Under such circumstances, glucose output from the liver and fatty acid release from adipose tissue are greatly enhanced. Adipocyte fatty acid esterification is restrained. The glucocorticoid effects are thus in good agreement with the different roles of PEPCK in liver and in adipose tissue. In adipose and hepatic cells, regulation by glucocorticoids is transcriptional, at least in part, and the promoter regulatory region of the gene is implicated [Franckhauser et al., 1995; Granner et al., 1991]. The mechanism underlying this complex feature of cell type–dependent opposite control of a single gene by glucocorticoids can be addressed by analyzing PEPCK gene transcriptional regulation.

Another potential way by which hormones can regulate gene expression is through modifications of mRNA half-life. In hepatoma cells, a stabilizing effect of cAMP on PEPCK mRNA has been extensively described, and the regulation of several RNA-binding proteins by cAMP has been reported [Hod and Hanson, 1988; Christ et al., 1991; Christ and Nath, 1993; Nachaliel et al., 1993]. The situation is the subject of controversy regarding glucocorticoids. Indeed, these agents have been reported to induce a stabilization of PEPCK mRNA [Petersen et al., 1989] and to have no effect [Hod, 1994].

In the present study, we have evaluated the potential posttranscriptional effect of glucocorticoids and of β-agonists in adipocytes. We demonstrate that, in addition to inducing transcriptional effect, β-agonists stabilize PEPCK mRNA, while the repressing effect of glucocorticoids is purely transcriptional. We show also that glucocorticoids act through different mechanisms to reduce basal PEPCK gene expression and to repress stimulation by β-agonists.

MATERIALS AND METHODS

Cell Culture

3T3-F442A cells were grown and differentiated exactly as described [Franckhauser et al., 1995]. All experiments were achieved on differentiated adipocytes. Dexamethasone or isoprenaline treatments were carried out in serum-free medium.

PEPCK mRNA Analysis

Total RNA was extracted using the method of Chomczynski and Sacchi [1987] and separated by agarose gel electrophoresis as described [Franckhauser et al., 1995]. After transfer onto a nylon membrane (Hybond N+; Amersham, Braunschweig, Germany), RNA was hybridized with a ^{32}P-labelled PEPCK cDNA probe (PC116) [Beale et al., 1985] or a 18S oligonucleotide [Franckhauser et al., 1995] using the Quick Hyb hybridization solution (Stratagene, La Jolla, CA) according to the manufacturer's recommendations. Membranes were exposed to X-ray film (XAR5; Kodak, Rochester, NY), and signals were quantified by scanning densitometry.

Stable Transfections and Analysis of CAT Activity

The generation of the FL15 stable transfectant has been described previously [Franckhauser et al., 1995]. Briefly, 3T3-F442A growing adipoblasts (1.7×10^3 cells/cm^2) were cotransfected with 2 µg of pSV2-NEO (SV40 promoter driving expression of the NEO gene which confers resistance to the antibiotic geneticin) (G418; Gibco Laboratories, Grand Island, NY) and 20 µg of pPL1-CAT (−2,100 to +69 bp of the PEPCK gene promoter fused to the chloramphenicol acetyltransferase (CAT) gene) by the calcium phosphate/DNA method. G418-resistant colonies were isolated and analyzed as described [Franckhauser et al., 1995]. CAT activity was measured on cell homogenates by the method of Seed and Sheen [1988]. One unit of CAT converts 1 µmole of chloramphenicol to butyryl-chloramphenicol per minute at pH 7.8 and 37°C.

RESULTS

Requirement for Protein Synthesis in Glucocorticoid and β-Agonist Action

We previously showed that β-agonists and glucocorticoids oppositely regulated PEPCK mRNA expression in 3T3-F442A adipocytes [Franckhauser et al., 1995]. To decipher the mechanisms by which these hormones modulated PEPCK mRNA, we treated 3T3-F442A adipocytes with dexamethasone or isoprenaline with or without various protein synthesis inhibitors. We chose cycloheximide, anisomycin, and puromycin, three inhibitors with different sites of action [Vazquez, 1979]. As expected from our previous studies [Franckhauser et al., 1995],

isoprenaline stimulated PEPCK mRNA about threefold in 4 h, whereas dexamethasone strongly reduced basal PEPCK mRNA and at least partially inhibited the isoprenaline-induced increase (Fig. 1A,B). Cycloheximide or anisomycin had no effect on basal PEPCK mRNA, while puromycin reduced it about twofold (Fig. 1B). This specific effect of puromycin might be the result of its site of action. Puromycin induces polysome dissociation, a mechanism which could favor RNA instability. None of these inhibitors affected isoprenaline induction or dexamethasone repression of isoprenaline stimulation. In contrast, the inhibitory action that dexamethasone exerted on basal PEPCK mRNA was prevented by the three translation inhibitors (Fig. 1B). Therefore, repression by glucocorticoids of basal PEPCK mRNA expression requires protein synthesis, whereas inhibition of induction by β-agonist is direct.

Time Course and Dose-Dependent Effects of Glucocorticoids on Induction of PEPCK mRNA by β-Agonists

We reported previously that glucocorticoids reduced basal PEPCK mRNA in 3T3-F442A adipocytes, with a maximum of 80% in 4 h [Franckhauser et al., 1995]. We wondered whether glucocorticoids would prevent isoprenaline induction in a similar time course. We treated 3T3-F442A adipocytes with isoprenaline and dexamethasone and compared the PEPCK mRNA signal to that obtained from cells incubated with isoprenaline alone. Dexamethasone prevented over at least 8 h the stimulation of PEPCK mRNA expression induced by isoprenaline (Fig. 2). We chose a 4 h treatment time to analyze the concentration dependence of the dexamethasone effect on the stimulation by isoprenaline. Dexamethasone produced a dose-dependent decrease in PEPCK mRNA with a half-maximal effect of at least 10 nM (Fig. 3), a concentration higher than the 1 nM value observed previously for the reduction of basal PEPCK mRNA [Franckhauser et al., 1995].

Transcriptional and Posttranscriptional Effects of Glucocorticoids and β-Agonists on PEPCK Gene Expression in 3T3-F442A Adipocytes

Using transcription run-on experiments, we demonstrated previously that β-agonist and glucocorticoid actions on PEPCK gene expression in 3T3-F442A adipocytes were transcriptional, at least in part. The observed changes of PEPCK mRNA may also be the consequence of a change in mRNA stability. To test this hypothesis, we measured the PEPCK mRNA half-life in hormone-treated adipocytes. For that purpose, we exposed 3T3-F442A adipocytes to the transcription inhibitor 5,6-dichloro-1β-ribofuranosyl benzimidazole (DRB) after a 1 h pretreatment with isoprenaline or dexamethasone. PEPCK mRNA concentration was followed as a function of time. In control cells PEPCK mRNA declined with a half-life of about 5 h (Fig. 4). Isoprenaline strongly stabilized PEPCK mRNA that reached a half-life of about 75 h. In contrast, dexamethasone did not affect PEPCK mRNA half-life in the basal or stabilized states. Hence, the glucocorticoid inhibitory effect appeared entirely transcriptional. To ascertain the transcriptional nature of the dexamethasone and β-agonist effects, we made use of a previously described 3T3-F442A stable transfectant, FL15, bearing −2,100 to +69 bp of the PEPCK gene promoter region fused to the CAT gene [Franckhauser et al., 1995]. FL15 cells were allowed to differentiate into adipocytes and then were treated for 10 h with dexamethasone or isoprenaline or both before measuring CAT. Variations in CAT activity directly reflect the hormonal modulation of the PEPCK promoter. As shown in Figure 5, dexamethasone reduced basal CAT expression by 40%. Isoprenaline stimulated CAT activity about 21-fold, and dexamethasone partly inhibited (60%) this induction. Hence, glucocorticoids and β-agonists clearly modulate transcription of the PEPCK gene and act via the promoter region of the gene.

DISCUSSION

In contrast to hepatocytes, adipocytes respond to glucocorticoids by a large decrease in PEPCK mRNA (Fig. 1) and gene transcription [Franckhauser et al., 1995] through a mechanism involving the glucocorticoid receptor [Franckhauser et al., 1994; Franckhauser-Vogel et al., 1997] and the 5′-flanking of the gene (Fig. 5). The absence of a dexamethasone effect on the PEPCK mRNA half-life in adipocytes (Fig. 4) demonstrates that the mechanism of glucocorticoid action is purely transcriptional. This fact is in good agreement with our former observation that the transcription rate of the gene is potently reduced by dexamethasone in the same cells [Franckhauser et al.,

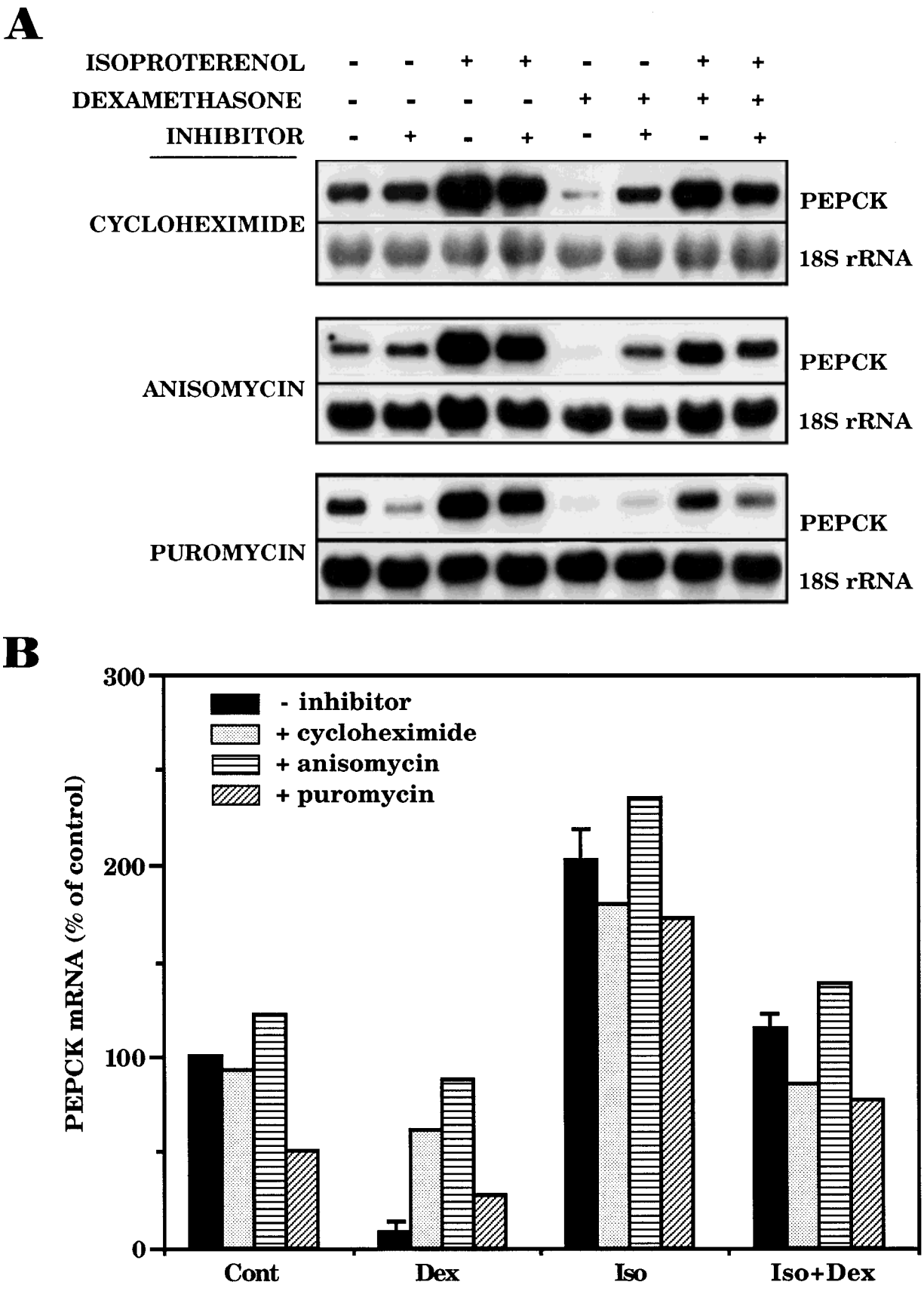

Fig. 1. Influence of translation inhibitors on hormonal control of PEPCK mRNA expression. Cells were pretreated 30 min with 10 µM cycloheximide, 30 µM anisomycin, or 1 mM puromycin and then treated 4 h with the same inhibitor and either 100 nM isoprenaline or 100 nM dexamethasone in serum-free medium. Total RNA was prepared and analyzed as described in Materials and Methods. **A:** Results of typical autoradiograms. **B:** Data presented in A were quantified relative to the corresponding 18S RNA signals by scanning densitometry. Values are in % of control, untreated cells.

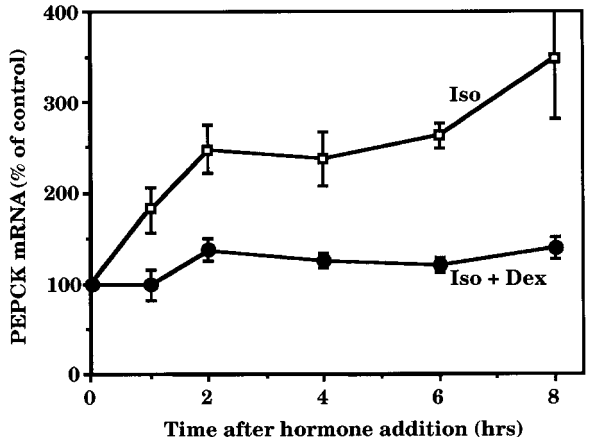

Fig. 2. Time course of glucocorticoid action on PEPCK mRNA induction by β-agonists. 3T3-F442A adipocytes were treated for various times with 100 nM isoprenaline alone (Iso) or simultaneously with 100 nM isoprenaline and 100 nM dexamethasone (Iso + Dex). Total RNA was prepared at the indicated times and analyzed as described in Materials and Methods. Results are presented in % of signal from nontreated cells (control). Data represent the mean ± s.e.m. of results obtained from three independent experiments.

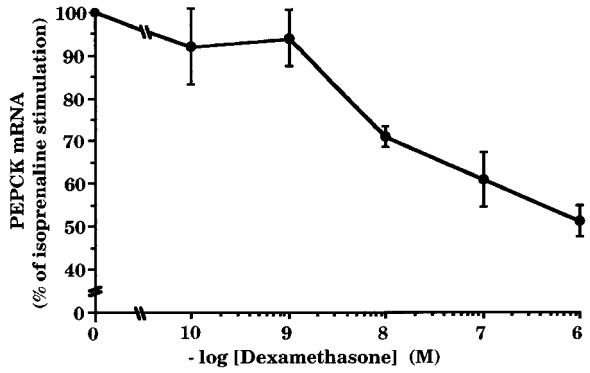

Fig. 3. Dose-dependent effect of glucocorticoids on PEPCK mRNA induction by β-agonists. 3T3-F4422A adipocytes were treated 4 h with 100 nM isoprenaline and various concentrations of dexamethasone. Total RNA was prepared and analyzed as described in Materials and Methods. Results are presented in % of signal from isoprenaline-treated cells. Data represent the mean ± s.e.m. of results obtained from three independent experiments.

1995]. In contrast to glucocorticoids, cAMP-producing agents like isoprenaline strongly stabilize PEPCK mRNA in 3T3-F442A adipocytes (Fig. 4). A similar situation occurs in hepatoma cells in which cAMP is also a stabilizer [Hod and Hanson, 1988]. Hence, in both cell types, cAMP exerts a posttranscriptional effect in addition to a well-characterized transcriptional stimulation (Fig. 5) [Lamers et al., 1982; Franckhauser et al., 1995; Sasaki and Granner, 1988]. Such a stabilizing effect of cAMP may be the

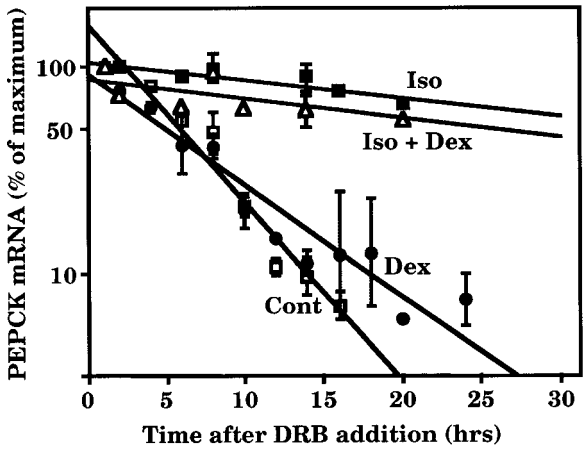

Fig. 4. Posttranscriptional action of dexamethasone and isoprenaline on PEPCK mRNA. 3T3-F442A adipocytes were maintained 36 h in serum-free medium before harvesting. Cells were pretreated for 1 h with no hormone (Cont), 100 nM dexamethasone (Dex), 100 nM isoprenaline (Iso), or both (Iso + Dex), and then 100 μM DRB was added for various times. Total RNA was prepared at the indicated times and analyzed as described in Materials and Methods. Data are presented as the percentage of signal at the time of addition of DRB and represent the mean ± s.e.m. of at least three independent experiments.

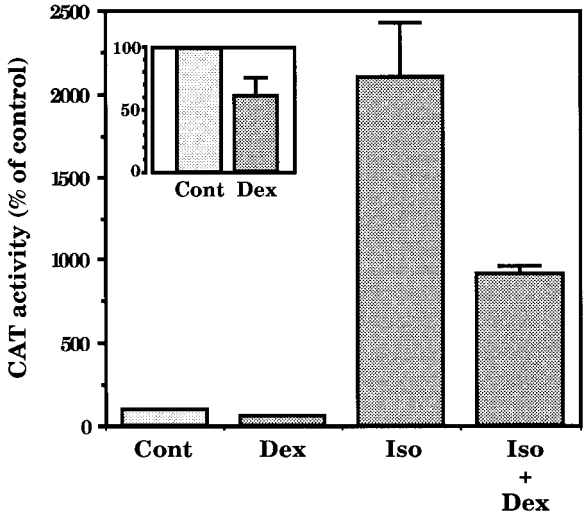

Fig. 5. Hormonal modulation of CAT activity in stably transfected PEPCK-CAT/3T3-F442A adipocytes. The stably transfected FL15 cells were allowed to differentiate 7 days as described [Franckhauser et al., 1995]. Adipocytes were then maintained 14 h in serum-free medium and treated for 10 h with no hormone (Cont), 100 nM dexamethasone (Dex), 100 nM isoprenaline (Iso), or both (Iso + Dex). CAT activity was measured on cell homogenates as previously described [Franckhauser et al., 1995]. Data are presented in % of control and represent the mean ± s.e.m. of results obtained from three independent experiments. **Inset:** Magnification of data obtained from control and dexamethasone-treated cells.

result of the interaction of RNA-binding proteins with the 3′-end of the PEPCK mRNA [Christ et al., 1991; Christ and Nath, 1993] or the inhibition by cAMP of a destabilizing protein [Nachaliel et al., 1993].

A surprising observation arises from our present study: the PEPCK mRNA half-life in 3T3-F442A adipocytes is about 5 h, a much longer time than the 50 min reported for hepatoma cells treated with DRB [Hod, 1994]. This would be consistent with the observation that PEPCK protein half-life is longer in adipose tissue than in liver [Hopgood et al., 1973]. However, a stabilizing effect of DRB on mRNAs has previously been described [Shyu et al., 1989]. If such an effect applies to the PEPCK mRNA, then the PEPCK mRNA half-life would be overestimated, providing an explanation for the observed 80% decrease produced by dexamethasone in 3T3-F442A adipocytes at 4 h of treatment (Fig. 1) [Franckhauser et al., 1995].

In addition to their lowering effect on basal PEPCK mRNA, glucocorticoids repress β-agonist induction with an equivalent time course (Fig. 2) [Franckhauser et al., 1995]. This repression is also purely transcriptional (Fig. 4) [Franckhauser et al., 1995] and involves the glucocorticoid receptor as determined by the inhibitory action exerted by the glucocorticoid antagonist RU 38486 [Franckhauser-Vogel et al., 1997]. However, two strong arguments indicate that different mechanisms mediate the reduction by glucocorticoids of basal and of isoprenaline-induced transcription. First, the concentration of dexamethasone required for producing the half-maximal effect shows a one order of magnitude difference (Fig. 3) [Franckhauser et al., 1995]. Second, inhibition of β-agonist–induced stimulation is direct, whereas reduction of basal transcription requires protein synthesis, suggesting that a glucocorticoid-induced transcription factor is involved in the latter mechanism (Fig. 1). This transcription factor could be the C/EBPδ isoform of the CCAAT enhancer binding protein (C/EBP) family because 1) it is rapidly induced by glucocorticoids in adipocytes [MacDougald et al., 1994], 2) C/EBP isoforms are implicated in the liver expression of PEPCK [Park et al., 1993], and 3) several C/EBP binding sites are present in the PEPCK promoter [Park et al., 1990; O'Brien et al., 1994]. Among the latter, the cAMP response element (CRE), the P3I sequence, and the accessory factor 2 element (AF2) appear potentially important. Indeed, in hepatic cells, the former two are implicated in the cAMP response and the latter in glucocorticoid stimulation [Park et al., 1990; Liu et al., 1991; Imai et al., 1990]. Moreover, the CRE is also an element involved in basal transcription in hepatoma cells [Quinn et al., 1988; Xing and Quinn, 1993]. If this situation also prevails in adipocytes, one could imagine that C/EBPδ would impede basal transcription.

The AF2 element is part of the complex region, the glucocorticoid response unit (GRU), the integrity of which is required for the full induction of the PEPCK gene by dexamethasone in hepatoma cells [Imai et al., 1990]. The GRU encompasses two glucocorticoid receptor binding sites (GREs) located around −400 bp of the promoter and two elements upstream of the GREs: an element named AF1 and the AF2 sequence. A sequence named AF3, located downstream of the GREs, recently has been shown to be an accessory factor element required for a complete glucocorticoid response [Scott et al., 1996]. The AF1 element is able to bind a retinoic X receptor (RXR) in heterodimer with either a retinoic acid receptor (RAR) or a peroxisome proliferator activated receptor (PPAR) [Hall et al., 1992; Tontonoz et al., 1995]. Two orphan members of the nuclear receptor family, hepatic nuclear factor-4 (HNF-4) and chicken ovalbumin upstream promoter-transcription factor (COUP-TF), also interact with the AF1 sequence and function as accessory factors in the hepatic glucocorticoid response [Hall et al., 1995]. However, 3T3-F442A adipocytes transiently transfected with pPL1-CAT in which the AF1 sequence has been inactivated by site-directed mutagenesis respond to dexamethasone with a repression of basal and of isoprenaline-induced CAT expression [Franckhauser-Vogel et al., 1997]. This shows that the AF1 element is not the target for the effect of glucocorticoids in adipocytes. Whether the GREs, AF2, or AF3 sequences are functional in adipocytes is unknown at present.

The adipocyte-specific inhibitory effect of glucocorticoids on the stimulation of PEPCK gene expression by β-agonists is independent of ongoing protein synthesis (Fig. 1). The observation that GR can interact physically with the CRE binding protein (CREB) [Imai et al., 1993] provides a potential mechanism by which glucocorticoids can repress β-agonist–induced transcription in a protein synthesis–independent fashion. In hepatoma cells, the CRE at about −90 bp is

implicated in the full positive response of the PEPCK gene to glucocorticoids [Xing and Quinn, 1993; Imai et al., 1993]. In a similar way, in AtT-20 cells the CRE present in the corticotropin-releasing hormone gene promoter mediates both positive cAMP regulation and glucocorticoid repression of cAMP-stimulated transcription probably via an interaction between GR and CREB [Guardiola-Diaz et al., 1996].

Glucocorticoids modulate transcription of many genes. Among these, an increasing number are shown to be under negative control, and the precise underlying mechanisms appear diverse, complex, and still unresolved. Our demonstration that transcription of the PEPCK gene is repressed by glucocorticoids in a cell-specific fashion and that reduction of basal transcription and inhibition of stimulation follow two different regulatory pathways highlights the suitability of this gene as a valuable model for deciphering the mechanisms of glucocorticoid negative action.

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REFERENCES

Beale EG, Chrapkiewicz NB, Scoble HA, Metz RJ, Quick DP, Noble RL, Donelson JE, Biemann K, Granner DK (1985): Rat hepatic cytosolic phosphoenolpyruvate carboxykinase (GTP). J Biol Chem 260:10748–10760.

Chomczynski P, Sacchi N (1987): Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159.

Christ B, Nath A (1993): The glucagon-insulin antagonism in the regulation of cytosolic protein binding to the 3′ end of phosphoenolpyruvate carboxykinase mRNA in cultured rat hepatocytes. Eur J Biochem 215:541–547.

Christ B, Heise T, Jungermann K (1991): Binding of cytosolic protein from cultured rat hepatocytes to the 3′-end of the phosphoenolpyruvate carboxykinase mRNA. Significance for protein-mediated mRNA stabilization. Biochem Biophys Res Commun 177:1273–1282.

Franckhauser S, Antras-Ferry J, Robin P, Robin D, Forest C (1994): Glucocorticoids antagonize retinoic acid stimulation of PEPCK gene transcription in 3T3-F442A adipocytes. Cell Mol Biol 40:723–729.

Franckhauser S, Antras-Ferry J, Robin P, Robin D, Granner DK, Forest C (1995): Expression of the phosphoenolpyruvate carboxykinase gene in 3T3-F442A adipose cells: Opposite effects of dexamethasone and isoprenaline on transcription. Biochem J 305:65–71.

Franckhauser-Vogel S, Glorian M, Forest C (1997): Glucocorticoids use a positive liver element to repress fibrate-induced adipose transcription of the PEPCK gene. Mol Cell Endocrinol 127:171–177.

Granner DK, O'Brien R, Imai E, Forest C, Mitchell J, Lucas P (1991): Complex hormone response unit regulating transcription of the phosphoenolpyruvate carboxykinase gene: From metabolic pathways to molecular biology. Rec Prog Horm Res 47:319–347.

Guardiola-Diaz HM, Kolinske JS, Gates LH, Seasholtz AF (1996): Negative glucocorticoid regulation of cyclic adenosine 3′,5′-monophosphate–stimulated corticotropin-releasing hormone-reporter expression in AtT-20 cells. Mol Endocrinol 10:317–329.

Hall RK, Scott DK, Noisin EL, Lucas PC, Granner DK (1992): Activation of the phosphoenolpyruvate carboxykinase gene retinoic acid response element is dependent on a retinoic acid receptor/coregulator complex. Mol Cell Biol 12:5527–5535.

Hall RK, Sladek FM, Granner DK (1995): The orphan receptors COUP-TF and HNF-4 serve as accessory factors required for induction of phosphoenolpyruvate carboxykinase gene transcription by glucocorticoids. Proc Natl Acad Sci U S A 92:412–416.

Hod Y (1994): The degradation of phosphoenolpyruvate carboxykinase (GTP) mRNA is regulated by cyclic AMP but not by dexamethasone. Arch Biochem Biophys 308:82–88.

Hod Y, Hanson R (1988): Cyclic AMP stabilizes the mRNA for phosphoenolpyruvate carboxykinase (GTP) against degradation. J Biol Chem 263:7747–7752.

Hopgood MJ, Ballard FJ, Reshef L, Hanson RW (1973): Synthesis and degradation of phosphoenolpyruvate carboxykinase in rat liver and adipose tissue. Changes during a starvation-refeeding cycle. Biochem J 134:446–452.

Imai E, Stromstedt PE, Quinn PG, Carlstedt-Duke J, Gustafsson JA, Granner DK (1990): Characterization of a complex glucocorticoid response unit in the phosphoenolpyruvate carboxykinase gene. Mol Cell Biol. 10:4712–4719.

Imai E, Miner JN, Mitchell JA, Yamamoto KR, Granner DK (1993): Glucocorticoid receptor-cAMP response element-binding protein interaction and the response of the phosphoenolpyruvate carboxykinase gene to glucocorticoids. J Biol Chem 268:5353–5356.

Lamers WH, Hanson RW, Meisner HM (1982): cAMP stimulates transcription of the gene for cytosolic phosphoenolpyruvate carboxykinase in rat liver nuclei. Proc Natl Acad Sci U S A 79:5137–5141.

Liu J, Park EA, Gurney AL, Roesler WJ, Hanson RW (1991): Cyclic AMP induction of phosphoenolpyruvate carboxykinase (GTP) gene transcription is mediated by multiple promoter elements. J Biol Chem 266:19095–19102.

MacDougald OA, Cornelius P, Lin FT, Chen SS, Lane MD (1994): Glucocorticoids reciprocally regulate expression of the CCAAT/enhancer-binding protein α and δ genes in 3T3-L1 adipocytes and white adipose tissue. J Biol Chem 269:19041–19047.

Meyuhas O, Reshef L, Gunn JM, Hanson RW, Ballard FJ (1976): Regulation of phosphoenolpyruvate carboxykinase (GTP) in adipose tissue in vivo by glucocorticoids and insulin. Biochem J 158:1–7.

Nachaliel N, Jain D, Hod Y (1993): A cAMP-regulated RNA-binding protein that interacts with phosphoenolpyruvate carboxykinase (GTP) mRNA. J Biol Chem 268: 24203–24209.

Nebes VL, Morris SM (1987): Induction of mRNA for phosphoenolpyruvate carboxykinase (GTP) by dexamethasone in cultured rat hepatocytes requires on-going protein synthesis. Biochem J 246:237–240.

O'Brien RM, Lucas PC, Yamasaki T, Noisin EL, Granner DK (1994): Potential convergence of insulin and cAMP signal transduction systems at the phosphoenolpyruvate carboxykinase (PEPCK) gene promoter through CCAAT/ Enhancer binding protein (C/EBP). J Biol Chem 269: 30419–30428.

Park EA, Roesler WJ, Liu J, Klemm DJ, Gurney AL, Thatcher JD, Shuman J, Friedman A, Hanson RW (1990): The role of the CCAAT/enhancer-binding protein in the transcriptional regulation of the gene for phosphoenolpyruvate carboxykinase (GTP). Mol Cell Biol 10:6264–6272.

Park EA, Gurney AL, Nizielski, Hakimi P, Cao Z, Moorman A, Hanson RW (1993): Relative roles of CCAAT/enhancer-binding protein β and cAMP regulatory element-binding protein in controlling transcription of the gene for phosphoenolpyruvate carboxykinase (GTP). J Biol Chem 268: 613–619.

Petersen D, Koch SR, Granner DK (1989): 3′ noncoding region of phosphoenolpyruvate carboxykinase mRNA contains a glucocorticoid-responsive mRNA-stabilizing element. Proc Natl Acad Sci U S A 86:7800–7804.

Quinn PG, Wong TW, Magnuson MA, Shabb JB, Granner DK (1988): Identification of basal and cyclic AMP regulatory elements in the promoter of the phosphoenolpyruvate carboxykinase gene. Mol Cell Biol 8:3467–3475.

Reshef L, Hanson RW, Ballard FJ (1969): Glyceride-glycerol synthesis from pyruvate. J Biol Chem 244:1994–2001.

Reshef L, Hanson RW, Ballard FJ (1970): A possible physiological role for glyceroneogenesis in rat adipose tissue. J Biol Chem 245:5979–5984.

Rongstad R (1979): Rate-limiting steps in metabolic pathways. J Biol Chem 254:1875–1878.

Sasaki K, Granner DK (1988): Regulation of phosphoenolpyruvate carboxykinase gene transcription by insulin and cAMP: Reciprocal actions on initiation and elongation. Proc Natl Acad Sci U S A 85:2954–2958.

Sasaki K, Cripe TP, Koch SR, Andreone TL, Petersen DD, Beale EG, Granner DK (1984): Multihormonal regulation of phosphoenolpyruvate carboxykinase gene transcription. J Biol Chem 259:15242–15251.

Scott DK, Mitchell JA, Granner DK (1996): The orphan receptor COUP-TF binds to a third glucocorticoid accessory factor element within the phosphoenolpyruvate carboxykinase gene promoter. J Biol Chem 271:31909–31914.

Seed B, Sheen JY (1988): A simple phase-extraction assay for chloramphenicol acyltransferase activity. Gene 67: 271–277.

Shyu AB, Greenberg ME, Belasco JG (1989): The c-fos transcript is targeted for rapid decay by two distinct mRNA degradation pathways. Genes Dev 3:60–72.

Tontonoz P, Hu E, Devine J, Beale EG, Spiegelman BM (1995): PPARγ2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. Mol Cell Biol 15: 351–357.

Vazquez D (1979): Inhibitors of protein synthesis. In Kleinzeller A, Springer GF, Wittman HG (eds): "Molecular Biology, Biochemistry and Biophysics," Vol 30. Berlin, Heidelberg, and New York: Springer-Verlag, pp. 63–79.

Xing L, Quinn PG (1993): Involvement of 3′,5′-cyclic adenosine monophosphate regulatory element binding protein (CREB) in both basal and hormone-mediated expression of the phosphoenolpyruvate carboxykinase (PEPCK) gene. Mol Endocrinol 7:1484–1494.