Expression of the phosphoenolpyruvate carboxykinase gene in 3T3-F442A adipose cells: opposite effects of dexamethasone and isoprenaline on transcription

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The enzyme phosphoenolpyruvate carboxykinase (PEPCK) plays a key role in gluconeogenesis in liver and in glyceroneogenesis in adipose tissue. These processes, and PEPCK, are regulated by a number of hormones, some of which have different effects on the enzyme in liver and adipose tissue. To explore this phenomenon, PEPCK gene expression was studied in 3T3-F442A adipocytes maintained in a serum-free medium. The β-adrenergic agonist isoprenaline (isoproterenol) and a cyclic AMP analogue (8-CPT-cAMP) increased PEPCK mRNA. A maximal 3-fold induction occurred in 2 h. Dexamethasone decreased PEPCK mRNA by 80% in 4 h. Dexamethasone also counteracted the inductive effects of isoprenaline and 8-CPT-cAMP. Run-on transcription experiments showed that the isoprenaline and dexamethasone actions were, at least in part, exerted at the level of PEPCK gene transcription. These effects were further analysed by using transient and stable transfection of adipocytes with a plasmid containing bp −2100 to 69 of the PEPCK gene promoter fused to the chloramphenicol acetyltransferase (CAT) gene. In such cells isoprenaline stimulated CAT expression, an effect that was prevented if the cells were also exposed to dexamethasone.

INTRODUCTION

Cytosolic phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32; PEPCK) catalyses a critical step in hepatic gluconeogenesis, the conversion of oxaloacetate into phosphoenolpyruvate. Expression of PEPCK is not restricted to the liver. Adipose tissue, kidney cortex, mammary gland and small intestine also express the enzyme to various extents [1]. It has been suggested that in adipose tissue PEPCK maintains glyceroneogenesis when the supply of glucose is limited [2]. The activity of the enzyme is not affected by post-translational modification, so modulations of enzyme activity are a reflection of the content of PEPCK mRNA in the cell. A variety of hormones affect PEPCK activity by altering transcriptional and post-transcriptional regulation of PEPCK mRNA.

Hormonal regulation of PEPCK gene expression has been mainly studied in liver, primary hepatocytes and cultured H4IIE rat hepatoma cells. In the last, cyclic AMP (cAMP), glucocorticoids (dexamethasone), retinoic acid and tri-iodothyronine stimulate PEPCK gene transcription, whereas insulin, phorbol esters (phorbol 12-myristate 13-acetate) and vanadate inhibit this process through *cis*-acting elements present in the promoter region of the gene [3–10]. Moreover, cAMP and dexamethasone increase PEPCK mRNA stability, whereas insulin destabilizes this mRNA [11–13]. These latter effects appear to be exerted through the 3′ untranslated region of the mRNA [12,13]. Thus regulation of PEPCK gene expression in liver involves a complex network of regulatory pathways.

The hormonal control of PEPCK gene expression is less well documented in adipose tissue. In intact rats, and in organ culture, noradrenaline stimulates PEPCK gene expression in adipose tissue, but insulin and glucocorticoids are inhibitory [14,15]. Moreover, when adrenalectomized fasted rats are treated with either dexamethasone or triamcinolone, transcription of the PEPCK gene is repressed in adipose tissue [15]. Although not demonstrated directly, one can thus expect that dexamethasone would inhibit transcription of the PEPCK gene in adipocytes. These different effects exerted by glucocorticoids in liver and adipose tissue are of particular interest, especially when one considers that this single-copy gene is under the regulation of a single promoter [15,16].

The PEPCK gene is expressed when preadipose cells differentiate into adipocytes [17–19]. cAMP increases and dexamethasone decreases PEPCK mRNA in differentiated 3T3-L1 cells [19]. Moreover, insulin and dexamethasone prevent the induction by cAMP [19]. Thus the regulation of the PEPCK gene appears to be similar in adipose tissue and in cultured adipocytes.

The aim of the present study was to characterize the mechanisms by which cAMP and dexamethasone modulate PEPCK gene expression in adipose cells. Using 3T3-F442A adipocytes as a model system, we now show that the β-adrenergic agonist isoprenaline increases PEPCK gene transcription whereas dexamethasone decreases transcription and inhibits, in a dominant manner, the isoprenaline-induced stimulation. We also show that the 5′ flanking region of the PEPCK gene is involved in both isoprenaline stimulation and in the inhibition of isoprenaline action by dexamethasone.

MATERIALS AND METHODS

Cell culture and hormone treatment

The 3T3-F442A cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing glucose (25 mM), penicillin (200 i.u./ml), streptomycin (50 mg/l), biotin (8 mg/l), pantothenate (4 mg/l), $NaHCO_3$ (3.7 g/l) and 10% (v/v) fetal-calf serum. They were grown at 37 °C in a humidified atmosphere of CO_2/air (1:9). Unless otherwise stated, preadipocytes (confluent

Abbreviations used: PEPCK, phosphoenolpyruvate carboxykinase; cAMP, cyclic AMP; DMEM, Dulbecco's modified Eagle's medium; GPDH, glycerophosphate dehydrogenase; CAT, chloramphenicol acetyltransferase; LPL, lipoprotein lipase.

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cells, referred to as day 0) were exposed to insulin (20 nM) to favour triacylglycerol accumulation, and the medium was changed every 2–3 days for 8 days. Before addition of hormone, the differentiated cells (day 8) were maintained in serum-free insulin-free medium for 24 h.

RNA extraction and analysis

Total RNA was extracted from cell monolayers by the method of Chomczynski and Sacchi [20]. For each treatment group, unless otherwise stated, RNA was isolated from cells pooled from two 6 cm-diameter dishes and was analysed by Northern-blot transfer as follows. Total RNA (20 μg) was electrophoresed in a 1%-agarose gel containing 2.2 M formaldehyde in 1 × MOPS buffer (40 × MOPS buffer contains: Mops, 0.8 M; sodium acetate, 0.2 M; EDTA, 0.04 M). The RNA was then transferred on to a Hybond-N membrane (Amersham Corp.) and linked by u.v. cross-linking. The integrity and relative amounts of RNA were assessed by Methylene Blue staining as described [21]. Specific mRNAs of interest were identified by hybridization with ^{32}P-labelled probes prepared by using the Multiprime labelling system kit (Amersham Corp.). Probes were: mL5, a mouse lipoprotein lipase cDNA [22]; GPDH-2e, a fragment containing the second exon and part of the second intron of the glycerophosphate dehydrogenase (GPDH) gene [23]; PC116, a rat PEPCK cDNA fragment [16]; and pAct1, a fragment of mouse actin cDNA [24]. Prehybridization and hybridization of the blots were performed with the Quick Hyb buffer (Stratagene) according to the manufacturer's instructions. Membranes were then washed for 2 × 15 min at room temperature with 2 × SSC (SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7)/0.1% SDS and 30 min at 60 °C with 0.1 × SSC/0.1% SDS. An oligonucleotide specific for the 18 S rRNA was ^{32}P-labelled and used as a control, as described previously [25]. Membranes were exposed to X-ray film for 4–48 h at −80 °C with intensifying screens. Quantification was performed by scanning densitometry. Hormonal treatments had no effect on β-actin mRNA or the 18 S rRNA concentrations. Thus the PEPCK mRNA signal was quantified relative to the corresponding actin mRNA signal.

Nuclear run-on transcription assay

The purification of nuclei and nuclear run-on experiments were done essentially as described by Antras et al. [26]. Briefly, nascent transcripts were elongated for 20 min at 30 °C with 250 μCi of [α-^{32}P]UTP (400 Ci/mmol; Amersham Corp.). Then (5–7) × 10^6 c.p.m. of the purified transcripts were hybridized to nitrocellulose filters previously bound with an excess of linearized cDNA or genomic probes (5 μg) for 72 h at 42 °C. The β-actin probe used was described above, and the PEPCK probe was a DNA fragment (.R3) of the PEPCK gene [16]. The filters were extensively washed, as described [26], and exposed to XAR5 Kodak film for about 24 h at −80 °C with intensifying screens. Quantification of the signals was performed by either densitometric scanning or scintillation counting of the nitrocellulose fragments, after standardization using the β-actin signal. Similar results were obtained with either method of quantification.

Transient and stable transfections

The PEPCK–CAT [pPLI-CAT; −2100 to +69 bp of the PEPCK gene promoter fused to the chloramphenicol acetyltransferase (CAT) gene], pSV$_2$-CAT (SV40 promoter fused to the CAT gene) and pSV$_2$-NEO (SV40 promoter fused to the NEO gene) have been described in detail [7,27]. Plasmids were prepared by two successive equilibrium centrifugations in CsCl/ethidium bromide gradients [21]. For transient transfections, 3T3-F442A adipocytes, 3–4 days post-confluency, were transfected in suspension by using the calcium phosphate co-precipitation method. Precipitates were prepared exactly as described previously [7]. Cells in 10 cm-diameter plates were detached with trypsin, spun down at 800 *g* for 5 min in serum-containing DMEM and resuspended in the calcium phosphate–DNA co-precipitate. After 15 min of contact at room temperature, the cell suspension was diluted with serum-containing DMEM and poured into new plates to give a confluent monolayer, i.e. a quantity equivalent to about 1.5 original plates per new plate.

After 6 h at 37 °C the medium was replaced with DMEM containing 20% dimethyl sulphoxide. After 3 min the cell monolayer was washed three times with DMEM, and then fresh differentiation medium, i.e. containing serum and insulin, was added. Medium was replaced the next day, and cells were further incubated for 2 days, after which hormonal treatments were performed for 15 h in serum-free medium.

In stable transfection assays, growing cells were transfected in suspension as indicated above, except that they were seeded at a density of 10^5 cells per 10 cm plate. Cells were then treated essentially as described previously [7]. Plasmids used for co-transfection were pPL1-CAT (20 μg) and pSV$_2$-NEO (2 μg), whose expression confers resistance to the antibiotic geneticin (G418; Gibco Laboratories). Under these conditions, the transfection efficiency was around 4×10^{-5}. After 2 weeks of G418 treatment (0.4 mg/ml), either individual or pooled (about 20) colonies were picked and analysed. About 90% of these colonies were able to differentiate. Of these, about 90% expressed CAT, and about 70% of CAT-expressing cell colonies showed a stimulation of CAT activity in response to treatment with 1 μM isoprenaline for 15 h. Colonies of cells that fulfilled these criteria were selected for further use.

CAT assay and primer extension analysis

CAT assays on cell homogenates were performed exactly as detailed previously [28]. One unit of CAT converts 1.0 nmol of chloramphenicol into chloramphenicol 3-acetate/minute at pH 7.8 and 37 °C.

For primer extension analysis, an oligonucleotide (CAT30; 5′-TAGCTTCCTTAGCTCCTGAAAATCTCGCCA-3′) was designed to be complementary to the portion of the CAT construct located between +81 and +111 bp relative to the transcription start site of the pPL-1 plasmid [27]. It was 5′ end-labelled with [α-^{32}P]ATP (> 3000 Ci/mmol; Amersham) to a specific radioactivity of about 10^9 c.p.m./μg as indicated [21]. Primer extension analysis was performed essentially as described [7], except that 20 μg of total RNA rather than poly(A)$^+$ RNA was used.

RESULTS

Influence of the differentiation state and of serum deprivation on the expression of adipocyte markers in 3T3-F442A cells

Preadipocytes (confluent cells) express some lipoprotein lipase (LPL) mRNA, but do not express GPDH or PEPCK mRNAs ([29], and Figure 1). As described previously [29], adipose-cell differentiation is characterized by the appearance of lipid droplets in the cytoplasm of the cells, a large increase in LPL mRNA, the induction of late marker genes (GPDH, PEPCK) and a decrease in β-actin mRNA (Figure 1). The presence of fetal-bovine serum in the medium is required for adipose-cell differentiation, and this may alter the specific response of a gene to a hormone. In order to study further the multihormonal regulation of PEPCK gene expression in 3T3-F442A adipocytes, we decided first to

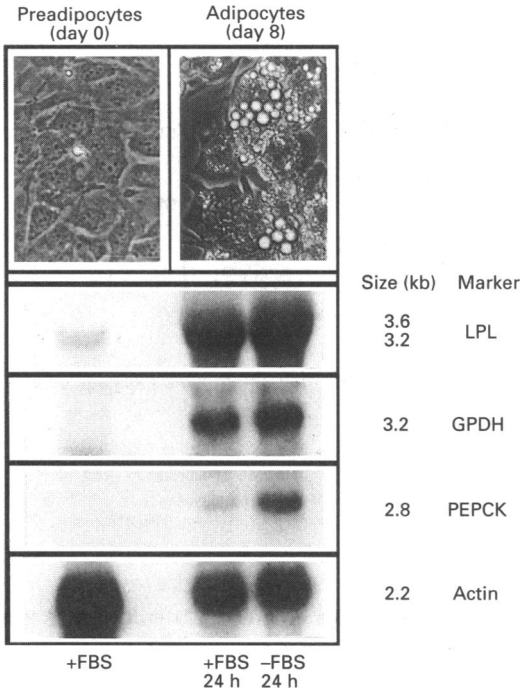

Figure 1 Influence of differentiation state and serum deprivation on the expression of various mRNAs in 3T3-F442A cells

Upper two panels: phase microscope photographs of preadipocytes, confluent at day 0, and adipocytes, (post-confluency, day 8); magnification ×240. Lower four panels: RNA was extracted from preadipocytes or adipocytes maintained under standard culture medium from which either insulin alone (+FBS, 24 h) or both insulin and fetal-bovine serum (−FBS, 24 h) were omitted for the last 24 h. Total RNA (20 μg) was analysed by blot transfer analysis. Labelled probes were used to detect LPL, GPDH, PEPCK and Actin mRNA. The autoradiograms were obtained by using the same blot with sequential hybridization. This result is representative of two separate experiments.

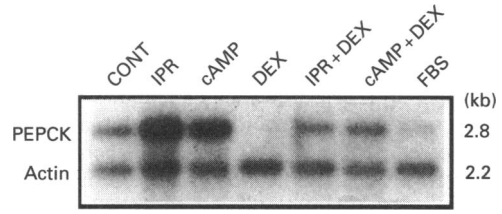

Figure 2 Multihormonal regulation of PEPCK gene expression in 3T3-F442A adipocytes

Differentiated adipocytes (day 8 post-confluency) were serum-deprived for 18 h, and then were treated with the indicated agents for 6 h: CONT, control untreated cells; IPR, isoprenaline (1 μM); DEX, dexamethasone (0.1 μM); cAMP, 8-(4-chlorophenylthio) cAMP (100 μM); FBS, fetal-bovine serum (10%). Total RNA (10 μg) was subjected to blot transfer analysis. The autoradiogram shown was obtained by first adding the actin probe and then the PEPCK probe. This result is representative of four separate experiments.

analyse the effect of serum on this gene. Serum deprivation for 24 h resulted in a 5-fold increase in PEPCK mRNA, without any change in LPL, GPDH or actin mRNAs (Figure 1). Conversely, when cells maintained for 24 h in the absence of serum were exposed for 6 h to 10% fetal-bovine serum, PEPCK mRNA decreased dramatically (Figure 2). Similar effects were obtained with different lots of bovine serum (results not shown). Since this

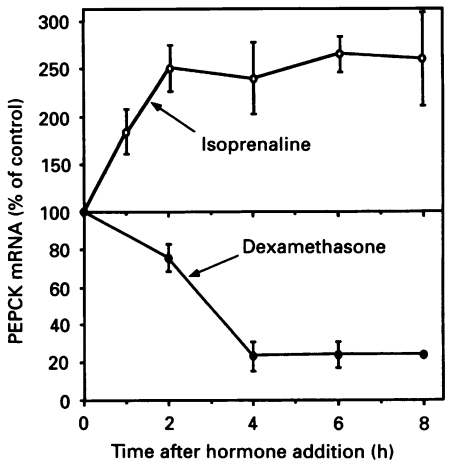

Figure 3 Time course of the effects of isoprenaline and dexamethasone on PEPCK mRNA in 3T3-F442A adipocytes

Differentiated adipocytes (day 8 post-confluency) were treated and RNA was analysed for PEPCK mRNA as indicated in the Materials and methods section. The isoprenaline and dexamethasone concentrations were 1 μM and 10 nM respectively. PEPCK mRNA was quantified by densitometry and the results are shown as percentage of the signal from control cells at zero time. Each value represents the mean ± S.E.M. of data obtained from three independent experiments.

effect of serum is as yet undefined, all subsequent experiments were performed using differentiated cells maintained in serum-free medium.

Hormonal modulation of PEPCK mRNA in 3T3-F442A adipocytes

The effects of isoprenaline, the cAMP analogue 8-(4-chlorophenylthio) cAMP or dexamethasone on PEPCK gene expression were tested in differentiated 3T3-F442A cells. A representative Northern blot is presented in Figure 2. When β-actin mRNA or 18 S rRNA was used as internal control, the level of PEPCK mRNA increased to 270 ± 17% in response to isoprenaline. A similar increase occurred in response to cAMP. PEPCK mRNA almost totally disappeared after dexamethasone treatment. Moreover, dexamethasone prevented the stimulation induced by isoprenaline or cAMP. These are not general effects, as S14 gene expression was stimulated by dexamethasone and was slightly inhibited by isoprenaline or cAMP ([30]; S. Franckhauser and C. Forest, unpublished work).

The effects of isoprenaline and dexamethasone were exerted rapidly (Figure 3). Isoprenaline stimulated a 2.5-fold increase of PEPCK mRNA in 2 h, whereas dexamethasone decreased PEPCK mRNA by almost 80% in 4 h. These new steady-state levels remained unchanged for at least 8 h. An exposure time of 2 h for isoprenaline and 4 h for dexamethasone was selected for determining the concentration-dependence of the hormone effects. In these experiments isoprenaline enhanced and dexamethasone decreased PEPCK mRNA in a concentration-dependent manner (Figure 4). The half-maximal effective concentrations were about 2 nM and 1 nM for isoprenaline and dexamethasone respectively.

Dexamethasone and isoprenaline modulate PEPCK-gene transcription in 3T3-F442A adipocytes

Two different approaches were used to determine whether the dexamethasone and isoprenaline effects are exerted at the level of

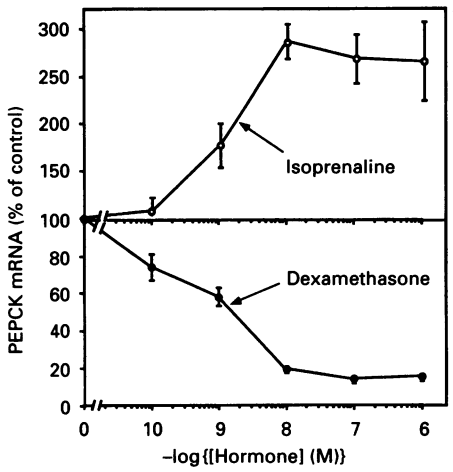

Figure 4 Effect of isoprenaline and dexamethasone concentrations on PEPCK mRNA in 3T3-F442A adipocytes

Differentiated adipocytes (day 8 post-confluency) were treated and the RNA was analysed as indicated in the Materials and methods section. Isoprenaline and dexamethasone treatments were for 2 and 4 h respectively. PEPCK mRNA was quantified as described in the legend to Figure 3. Each value represents the mean ± S.E.M. of data obtained from three independent experiments.

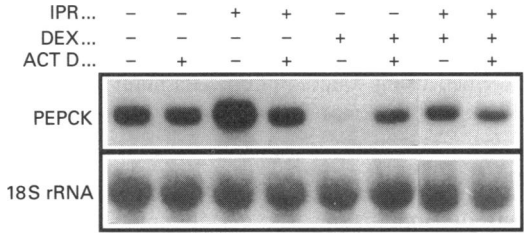

Figure 5 Influence of actinomycin D on the hormonal modulation of PEPCK gene expression in 3T3-F442A adipocytes

Differentiated adipocytes (day 8 post-confluency) were serum-deprived for 20 h, and then preincubated with or without actinomycin D (ACT D; 4 μg/ml) for 30 min. The cells were then treated with isoprenaline (IPR; 1 μM) and/or dexamethasone (DEX; 0.1 μM) for 4 h in the presence or absence of actinomycin D. Total RNA (20 μg) was subjected to blot transfer analysis. Autoradiograms were obtained with the same blot hybridized sequentially with the PEPCK and 18 S rRNA probes. This autoradiogram is representative of two separate experiments.

PEPCK gene transcription. In one test, cells were treated with actinomycin D to block transcription, and then exposed to various combinations of hormones (Figure 5). Actinomycin D had no effect on PEPCK mRNA in untreated cells, but it totally abolished the isoprenaline-induced increase and the dexamethasone-induced decrease in PEPCK mRNA. Such results indicate that isoprenaline and dexamethasone may modulate the transcription rate of the PEPCK gene. However, this approach is quite indirect, and actinomycin D itself may have side effects, such as, for example, a modification of the stability of some RNAs [11]. Thus the results obtained with the use of this inhibitor may lead to incorrect interpretations. Therefore, we decided to employ a second, more direct, approach, the nuclear run-on transcription experiment. The rate of PEPCK gene transcription increased slightly after a 2 h treatment of differentiated cells with isoprenaline (Figure 6). When the β-actin signal was used as an internal control, the increase by isoprenaline

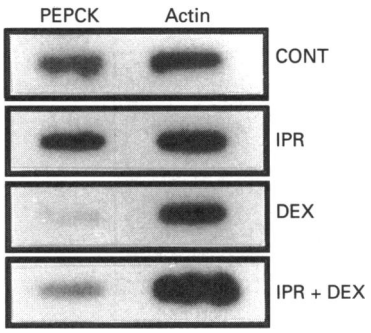

Figure 6 Hormonal regulation of the PEPK gene transcription rate in 3T3-F442A adipocytes

Differentiated cells (day 8) were serum-deprived for 22 h, and then treated or not (control; CONT) for 2 h with isoprenaline (IPR; 0.10 μM), dexamethasone (DEX; 0.1 μM) or both. Nuclei were then prepared and used for the transcription assay *in vitro* as described in the Materials and methods section. This autoradiogram is representative of two separate experiments.

Table 1 Hormonal modulation of CAT activity in transiently transfected 3T3-F442A adipocytes

Cells were transfected with either pPL1-CAT (20 μg/plate) or a mixture of pSV2-CAT (5 μg/plate) and pUC18 (15 μg/plate) as described in the Materials and methods section. Cells were then treated or not with dexamethasone (DEX; 0.1 μM), isoprenaline (IPR; 0.1 μM) or both for 15 h in serum-free medium. CAT activities from untreated cells and fold inductions by hormones are presented. Values represent means ± S.E.M. of data obtained from three independent experiments: $^*P < 0.05$ (Student's t test for paired data) compared with pPL1-CAT stimulated by isoprenaline.

Plasmid construct	Basal CAT activity (m-units/mg of protein)	Induction (fold)		
		IPR	DEX	IPR + DEX
pPL1-CAT	0.4 ± 0.1	5.1 ± 1.1	1.2 ± 0.2	3.1 ± 0.9*
pSV2-CAT	2.9 ± 1.1	1.4 ± 0.2	0.9 ± 0.2	1.2 ± 0.6

of PEPCK gene transcription was 1.6- and 1.8-fold for two independent experiments. Conversely, dexamethasone exposure resulted in a decrease of up to 80% in PEPCK gene transcription after 2 h. Moreover, dexamethasone prevented the isoprenaline-induced increase. Thus modulation of PEPCK gene expression by isoprenaline and dexamethasone operates, at least in part, at the level of the transcription rate of the gene.

Involvement of the PEPCK-gene regulatory region in the dexamethasone and isoprenaline effects

In H4IIE rat hepatoma cells, cAMP and dexamethasone modulate PEPCK gene transcription through *cis*-acting sequences present in the 5′ flanking region [3,4]. In order to begin to elucidate whether similar elements are involved in isoprenaline and dexamethasone action in adipocytes, we performed transient and stable transfections of 3T3-F442A cells, using a plasmid construct, pPL1-CAT, that contains the sequence of the PEPCK promoter from −2100 to +69 bp ligated to the CAT gene [27]. Adipocytes transiently transfected with this construct had an ~ 5-fold increase in CAT activity after 15 h of isoprenaline treatment in serum-free medium (Table 1). Dexamethasone had no effect on basal CAT activity, but it partially inhibited (40%) the isoprenaline stimulation. These effects were specific to pPL1-

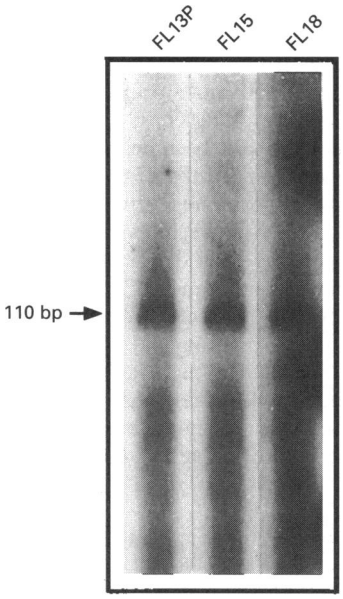

Figure 7 Primer extension analysis of pPLI-CAT expression in stable transfectants

Total RNA (20 μg) isolated from cells stably transfected with FL13Pool, FL15 and FL18 was used for primer extension analysis as described in the Materials and methods section. The result of a typical autoradiogram is shown. The correct size (110 bp) of the extended CAT primer is indicated by an arrow.

Table 2 Hormonal modulation of CAT expression in stably transfected cell lines

Two stably transfected cell lines (FL15 and FL18) and a pool of about 20 stably transfected cells (FL13Pool) were obtained by co-transfection of pPL1-CAT and pSV2-NEO as described in the Materials and methods section. Adipocytes (day 8 post-confluency) were treated or not with isoprenaline (IPR; 0.1 μM), dexamethasone (DEX; 0.1 μM) or both for 15 h in serum-free medium. CAT activities from untreated cells and fold inductions by hormones are presented. Values represent means ± S.E.M. of data obtained from three to five independent experiments: $^{**}P < 0.01$ and $^{*}P < 0.05$ (Student's t test for paired data) compared with isoproterenol stimulation.

Transfectant	Basal CAT activity (m-units/mg of protein)	Induction (fold)		
		IPR	DEX	IPR + DEX
FL15	5.1 ± 1.1	11.5 ± 1.3	1.2 ± 0.3	4.3 ± 1.0**
FL18	15.7 ± 3.8	2.1 ± 0.3	0.8 ± 0.1	0.7 ± 0.2*
FL13Pool	5.1 ± 1.2	2.0 ± 0.1	1.1 ± 0.2	1.3 ± 0.2*

CAT, as neither isoprenaline nor dexamethasone affected CAT activity in cells transiently transfected with the pSV2-CAT (SV40 promoter linked to the CAT gene) control plasmid (Table 1).

Several stably transfected cell lines were obtained by co-transfecting 3T3-F442A adipoblasts with pPLI-CAT and pSV2-NEO (SV40 promoter linked to the NEO gene) and selecting for NEO-resistant colonies with G418. Two stable colonies (FLI5 and FLI8) and one stable pool of about 20 colonies (FL13Pool) were analysed in detail. These three stably transfected cell lines differentiate at a frequency comparable with the parental 3T3-F442A line, all express the CAT gene, and primer extension analysis showed that PEPCK-CAT gene transcription initiates at the correct site in each (Figure 7). As expected from previous results [7], there was no correlation between basal CAT expression and the number of integrated copies of the gene (results not shown). After differentiation, stable transfectants responded to isoprenaline with a 2–11.5-fold increase in CAT activity (Table 2) that was linear for at least 20 h after hormone addition (results not shown). Dexamethasone had no effect on basal activity, but inhibited the isoprenaline-provoked increase (Table 2). Inhibitions by dexamethasone of isoprenaline inductions were of about 70%, 100% and 70% for FL15, FL18 and FL13Pool respectively (Table 2). Thus the PEPCK promoter from −2100 to +69 bp mediates the stimulation of the gene by isoprenaline and the inhibition by dexamethasone of isoprenaline action.

DISCUSSION

We used 3T3-F442A cells to analyse the mechanisms by which the β-agonist isoprenaline (or its intracellular mediator, cAMP) and the glucocorticoid analogue, dexamethasone, modulate PEPCK gene expression in adipocytes. Isoprenaline or cAMP increases PEPCK mRNA in these adipocytes, whereas dexamethasone decreases it. Moreover, dexamethasone inhibits the stimulation induced by isoprenaline or cAMP (Figure 2). The action of dexamethasone is thus very different in adipocytes from that in liver or hepatoma cells, wherein glucocorticoids stimulate PEPCK gene transcription.

The stimulatory effect that isoprenaline exerts on PEPCK mRNA is rapid (maximum in 2 h; see Figure 3), and it is exquisitely sensitive to isoprenaline, as the EC_{50} is about 2 nM, a value close to that required for the lipolytic action of this hormone in differentiated cells from preadipose lines [33,34]. The second messenger cAMP exerts the same stimulatory action as isoprenaline, suggesting that the effect of this agent is the consequence of its binding to a β-adrenergic receptor. We show that the isoprenaline effect is, at least in part, transcriptional (Figure 6) and involves the region between −2100 and +69 bp of the gene (Tables 1 and 2). This result indicates that in adipocytes, as in hepatoma cells, cAMP-response element(s) (CREs) are present in that region of the gene. In hepatoma cells, cAMP stimulates PEPCK gene transcription through at least two *cis*-acting elements located between −250 and −234 bp and between −92 and −82 bp relative to the transcription initiation site [3]. Whether the same elements are involved in cAMP action in adipocytes remains to be determined. Experiments designed to address this question are rendered difficult by the presence upstream of the liver CREs of a region between −2088 and −888 bp required for the expression of a PEPCK–human growth hormone gene construct in adipose tissue of transgenic mice [35]. This region lies outside the liver hormone-response domain. Moreover, we showed recently that the fragment of the PEPCK promoter between −600 and +69 bp is ineffective in driving expression of the CAT gene in stably transfected 3T3-F442A adipocytes [36]. Taken together, these results imply that, if the same CREs are involved in the response to cAMP of both liver and adipose tissue, additional element(s) located upstream of these are required for the gene to be activated in adipocytes.

The physiological relevance of cAMP stimulation of PEPCK gene expression in both liver and adipose tissue is consistent with the postulated roles played by this enzyme in these tissues. Starvation results in increased cAMP synthesis in both tissues, and this results in a stimulation of glucose production by the liver and fatty acid re-esterification in adipose tissue in order to restrain fatty acid output [2]. PEPCK has a key role in these two processes [1,2]. The observation that glucocorticoids regulate PEPCK gene expression positively in liver and negatively in adipose tissue is intriguing. The activity and rate of synthesis of

adipose-tissue PEPCK is increased by adrenalectomy and starvation [14]. The rise in plasma non-esterified fatty acids noted during starvation of rats is impaired by adrenalectomy and restored by treatment with glucocorticoids [37]. The addition of glucocorticoids to incubated adipose tissue increases fatty acid release and decreases fatty acid re-esterification [37]. Moreover, glucocorticoids are stress hormones, and stress is a situation during which energy must be available to tissues such as muscle. Under such circumstances, fatty acid release from adipose tissue is greatly enhanced and fatty acid esterification is restrained. The glucocorticoid effect is thus in good agreement with the postulated glyceroneogenic role of PEPCK in adipose tissue.

Here we show that dexamethasone rapidly and strongly decreases basal PEPCK gene expression in 3T3-F442A adipocytes (Figures 3 and 4). Half-maximum inhibition occurs at about 1 nM, a value close to the K_d of the binding of dexamethasone to the glucocorticoid receptor in these cells [38] or in isolated adipocytes [37]. Of interest is the observation that serum is as potent as dexamethasone in decreasing PEPCK mRNA level (Figures 1 and 2). Serum is a complex mixture of hormones, nutrients and growth factors. Among these, glucocorticoids may be the active component for PEPCK gene repression. However, our results do not sustain such an hypothesis, as S14 gene expression is not affected by serum deprivation for 6 or 24 h (results not shown), whereas dexamethasone is a potent activator of the transcription of that gene [30]. The nature of the serum effector is still unknown.

Whereas the repression of PEPCK mRNA by dexamethasone appears to be exerted at the level of transcription (Figure 6), dexamethasone has no effect on basal CAT activity in 3T3-F442A cells transiently or stably transfected with pPL1-CAT (Tables 1 and 2). Additional elements outside the −2100 to +69 bp PEPCK construct may be required for the dexamethasone effect in adipocytes. This would be consistent with the reported lack of effect *in vivo* of dexamethasone on the same PEPCK promoter fragment in adipose tissue of transgenic mice [39], although this result is a matter of debate [40]. In hepatoma cells, glucocorticoids enhance PEPCK gene transcription through a multicomponent regulatory complex (GRU) located between −455 and −349 bp [4]. The GRU contains two accessory factor elements (AF1 and AF2) and two glucocorticoid-receptor-binding sites (GR1 and GR2). Our results suggest that the GRU is not sufficient for dexamethasone to decrease PEPCK gene transcription in adipocytes. However, dexamethasone prevents isoprenaline induction of CAT expression in adipocytes transiently or stably transfected with pPL1-CAT (Tables 1 and 2), and this effect may be mediated by the GRU. Functional assays in adipocytes of modified pPL1-CAT containing either deletions or block mutations of the GRU defined in H4IIE cells are required to address this question.

A composite element has also been described in the regulation of the proliferin gene by glucocorticoids [41,42]. In that case, the glucocorticoid receptor and AP1, a transcription factor comprised of *c-jun* homodimers or *c-jun*/*c-fos* heterodimers, both recognize a 25 bp sequence in the promoter. In the absence of AP1, dexamethasone has no effect on the proliferin gene. In the presence of AP1, the relative proportion of c-jun and c-fos determines the direction of the effect [41,42]. A mechanism of this type can be proposed in the case of the PEPCK gene. The expression or the regulation of the AF1 and AF2 accessory factors may be tissue-specific and may modulate PEPCK gene transcription differently, depending on the cell context. Because of the mechanism of dexamethasone action in adipocytes, an interference between the glucocorticoid receptor and CREB (CRE-binding protein) can also be suggested. Of particular interest is the recent observation that the basal promoter/cyclic AMP response element (E/CRE) at about position −90 is involved in the full response of the gene to glucocorticoids [41]. This element binds CREB, and a protein–protein interaction has been observed *in vitro* between glucocorticoid receptor and CREB [43]. Situations have been described in which communication exists between transcription factors and various members of the steroid/thyroid hormone receptor family [42–45]. In some cases, direct interaction of the transcription factor AP1 with a particular receptor have been demonstrated [42]. For instance, the glucocorticoid receptor and AP1 can reciprocally repress each other's transcriptional activation by a mechanism that is independent of DNA binding [42]. Thus the glucocorticoid-receptor/CREB interaction might account for the role of the E/CRE in the glucocorticoid response of the PEPCK gene both in hepatocytes and in adipocytes, but with opposite effects in these cell types. We are at present conducting experiments to clarify this point.

We are indebted to Dr. Howard Green for kindly providing us with the 3T3-F442A cell line. We thank Dr. Bruce Spiegelman, Dr. Leslie Kozak and Dr. Michael Schotz for their gifts of the pAct-1 and pAD-5, pGPDH-2e, and pmL5 plasmids respectively. We also thank Sandrine Benac and Deborah Caplenor for their skilful secretarial assistance. This research was supported by INSERM CRE 91-0706 (C.F.) and by U.S. Health and Human Services Grants DK35107 (D.K.G.) and DK20593 (the Vanderbilt Diabetes Research and Training Center). J.A.-F. is a recipient of a fellowship from the Ligue Nationale Contre le Cancer.

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Received 18 May 1994/11 July 1994; accepted 26 July 1994