Phorbol esters inhibit the glucocorticoid-mediated stimulation of cytosolic aspartate aminotransferase gene transcription

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The regulation of cytosolic aspartate aminotransferase (cAspAT) gene expression by phorbol esters was investigated in the highly differentiated hepatoma cell line Fao. Phorbol 12,13-dibutyrate (PdBu) had no effect on basal activity but partially inhibited the induction of cAspAT by dexamethasone. The extent of inhibition (40 %) was similar to that obtained with insulin or vanadate. The inhibitory effects of PdBu and vanadate were additive. In the case of PdBu, the inhibitory effects could be eliminated by first incubating the cells with PdBu, which down-regulates protein kinase C. In contrast, inhibition by insulin was not modified by this treatment. The molecular mechanism of PdBu action was

investigated. Northern blot analysis showed that the steady-state mRNA levels of cAspAT were decreased by PdBu in the presence of dexamethasone. In addition, the transcription rate, as measured by run-on experiments, was also decreased under the same conditions. Finally, a 2.4 kb promoter fragment driving the chloramphenicol acetyltransferase gene was stably transfected into the Fao cells. The regulation of the activity of this promoter fragment by dexamethasone and PdBu was similar to the regulation of the endogenous cAspAT activity. We conclude that PdBu acts by regulating the promoter activity of the cAspAT gene.

INTRODUCTION

Cytosolic and mitochondrial aspartate aminotransferases (cAspAT and mAspAT) are ubiquitous enzymes involved in several metabolic pathways in the cell [1]. One of these critical pathways is the malate–aspartate shuttle, which leads to a net transport of reducing equivalents from the cytosol to the mitochondria [2]. Furthermore, these enzymes participate in gluconeogenesis and ureogenesis [2,3]. An interesting feature of these metabolic pathways is that some are constitutive and ubiquitously expressed, whereas others are hormonally regulated and tissue-specific. The expression of cAspAT is in keeping with its complex metabolic functions. Although it is expressed at a basal level in all tissues, it is regulated by glucocorticoids in the liver and kidney [4], and by glucagon only in the liver [3]. Furthermore, diabetes increases cAspAT activity in the liver [5].

We carried out a detailed analysis of the hormonal regulation of cAspAT in the highly differentiated hepatoma cell line Fao, and observed both positive and negative regulation. Glucocorticoids induced the cAspAT activity and the corresponding mRNAs [6]; this effect was potentiated by cyclic AMP and inhibited by insulin [6]. This type of regulation is consistent with the participation of cAspAT in gluconeogenesis. Indeed, similar regulation has been reported for phosphoenolpyruvate carboxykinase (PEPCK), a gluconeogenic enzyme which is also induced by glucocorticoids and cyclic AMP and inhibited by insulin [7]. It differs from cAspAT in that its basal expression is not ubiquitous but is restricted to certain tissues [8].

The tumour-promoting phorbol esters exert a wide variety of effects in several cell types [9]. They bind to, and activate, protein

kinase C (PKC), which is physiologically activated by diacylglycerol [10]. Phorbol esters modify the activity of several enzymes at the pre- or post-translational level [9]. The expression of several genes is altered by these compounds. Transcription of the *c-fos* [11] and ornithine decarboxylase [12] genes is increased, while that of the PEPCK gene is decreased [13]. The molecular mechanisms by which phorbol esters increase gene expression have been thoroughly investigated [14]. Most of its effects are mediated by the AP1 transcription complex [15] which is composed of the *fos* and *jun* proto-oncogene products [16]. The mechanisms by which gene expression is inhibited by phorbol esters are less well understood.

An interesting aspect concerning the effects of phorbol esters is their similarity to the effects of insulin [17,18]. It has been suggested that several of the effects of insulin are mediated by PKC [9]. However, several reports have questioned this hypothesis [12,19,20]. Alternatively, different cascades of events could converge at a common point downstream of PKC and lead to similar physiological regulation. This model has been proposed to explain the negative regulation of PEPCK by the same regulatory element in the case of both insulin and phorbol esters [21].

In order to further elucidate the effects of phorbol esters on gluconeogenic enzymes and, more generally, on enzymes involved in energy metabolism, we have studied the regulation of cAspAT by phorbol esters in the Fao hepatoma cell line. Our data show for the first time that phorbol esters inhibit the glucocorticoid-mediated induction of cAspAT by acting on the gene promoter. Furthermore, this negative effect of phorbol esters can be distinguished from the negative regulation exerted by insulin.

Abbreviations used: cAspAT, cytosolic aspartate aminotransferase; mAspAT, mitochondrial aspartate aminotransferase; PdBu, phorbol 12,13-dibutyrate; PEPCK, phosphoenolpyruvate carboxykinase; PKC, protein kinase C; PKA, protein kinase A; IRS, insulin-responsive sequence; TAT, tyrosine aminotransferase; CAT, chloramphenicol acetyltransferase; PMA, phorbol 12-myristate 13-acetate.

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EXPERIMENTAL

Materials

Dexamethasone, phorbol 12,13-dibutyrate (PdBu) and insulin were purchased from Sigma.

Cell culture

The rat hepatoma clone Fao is derived from line H₄IIEC₃, which was isolated from the Reuber H35 hepatoma [22]. Cells were propagated in a modified Ham's F12 medium (Gibco) supplemented with 5% calf serum (Flow), 100 units/ml penicillin, 100 µg/ml streptomycin (Lab. Diamant) and 0.5 µg/ml Fungi-zone (Squibb) [23]. Cells were cultured as monolayers on Falcon plastic Petri dishes at 37 °C in a humidified atmosphere (95% air/5% CO₂). The culture medium was changed three times per week. At each passage, cells were harvested with 0.05 g/100 ml trypsin and 0.2 mg/ml EDTA (Gibco).

Hormone treatment and preparation of the cells

Petri dishes (10 cm diameter) were inoculated with 2×10^6 cells and were treated with the various hormones and drugs. The medium was not changed during the treatment period. Before enzyme assays, the culture medium was removed, the cells were washed once with 0.01 M Tris/HCl, pH 7.3, and 0.27 M sucrose, detached with a scraper (Corning), centrifuged at 300 g and resuspended in 500 µl of 0.01 M Tris/HCl, pH 7.3, and 0.27 M sucrose.

AspAT assay

For the separate determination of cAspAT and mAspAT, we used the procedure of Parli et al. [23], which is based on the thermolability of mAspAT but not cAspAT. Triton X-100 (0.1%), 2-oxoglutarate (3.7 mM) and Tris/acetate (0.1 M), pH 7.4, were added to the homogenate, and the mixture was incubated either at 37 °C (total AspAT assay) or at 70 °C (cAspAT assay) for 15 min. The reaction mixture was then centrifuged at 10000 g for 20 min and the resulting supernatant was assayed for AspAT activity using a kit from Roche. The assay, carried out at 23 °C, was optimized by adding 0.1 mM pyridoxal phosphate to the supernatant 5 min prior to the assay. mAspAT activity was deduced by subtracting the cAspAT activity from the total activity. One unit is the amount of enzyme that catalyses the formation of 1 µmol of product/min at 23 °C. Protein was determined by the method of Lowry et al. [24]. The basal activity of cAspAT varied from one preparation of cells to the other; it tended to decrease as the passage number increased.

RNA preparation

Total RNA was isolated from cultured cells using the guanidium thiocyanate extraction method described by Chirgwin et al. [25]. Briefly, 40×10^6 cells were homogenized in 6 ml of guanidium thiocyanate using an Ultraturax. RNA was then extracted by three successive ethanol precipitations in acetic acid. The final RNA pellet was resuspended in 50 µl of water.

Northern blot hybridization

Total RNA (20 µg) was electrophoresed on a horizontal 1.2% agarose/2.2 M formaldehyde gel and transferred to a nylon membrane (Hybond N⁺; Amersham). The membrane was prehybridized for 24 h at 42 °C in 50% formamide, 5×SSC

(1×SSC = 0.15 M NaCl, 0.015 sodium citrate), 1×Denhardt's solution, 50 mM sodium phosphate, pH 6.8, 0.2% SDS and 200 µg/ml denatured salmon sperm DNA, and then hybridized overnight at 42 °C in the same medium containing 10% dextran sulphate and 2×10^6 c.p.m./ml denatured cDNA probe. A rat cAspAT DNA was isolated previously from a rat liver cDNA library in λgt11 [4]. The membrane was washed in 0.1×SSC/0.1% SDS at 68 °C to reduce background, and autoradiographed for 24–72 h at –80 °C using an intensifying screen and Amersham Hyperfilms MP. The intensities of the bands were determined by scanning densitometry. Northern blots were dehybridized as previously described and then rehybridized with a tyrosine aminotransferase (TAT) probe. The TAT cDNA was kindly donated by Dr. Gunther Schütz (German Cancer Research Center, Heidelberg, Germany) [26].

Statistical analysis

Statistical analysis of the enzyme activity determinations was done using the Mann-Whitney U test and the Student *t* test.

Run-on experiments

Nuclei were prepared as previously described [27]. Nascent RNA transcripts were labelled with [α -³²P]UTP, isolated and quantified according to the technique of Vaultont et al. [28] with minor modifications [29]. The labelled RNA (6×10^6 c.p.m.) was hybridized to Hybond nylon filters (Amersham) containing 10 µg of the linearized plasmid 4B21 containing the cAspAT cDNA [4], the linearized tubulin cDNA plasmid, the linearized 28 S ribosomal cDNA plasmid or the linearized vector (PGEM 3Z) alone, according to Antras et al. [29]. The nylon filters were washed at 65 °C for 1 h once in 2×SSC and once in 1×SSC, and then treated for 30 min at 37 °C in 1×SSC containing 10 µg/ml RNAase A. The final wash was carried out in 1×SSC for 1 h at 37 °C. Autoradiography of the filters was then performed using Amersham Hyperfilm MP and the resulting signal was quantified by densitometric analysis.

Plasmids

The various plasmids were constructed by inserting the desired fragment of the cAspAT gene promoter into the polylinker of pMW-1, a pUC18 derivative carrying the chloramphenicol acetyltransferase (CAT) reporter gene, as reported previously [30]. The p–2405/–26CAT construct was obtained by subcloning the *KpnI/XhoI* fragment of the cAspAT gene promoter in front of the CAT gene. The pSV2neo plasmid was described elsewhere [31].

Transfection experiments

At 1 day prior to the transfection, Fao cells (1.5×10^6 cells/10 cm dish) were seeded into the usual culture medium containing fetal calf serum; 10 ml of fresh medium containing serum was added to the cells 2–3 h before the transfection. The expression plasmid (10 µg) and the pSV2neo plasmid (2 µg) for permanent transfections were introduced into the cells by the calcium phosphate coprecipitation technique followed by a glycerol shock [32]. A 10 ml portion of fresh medium containing serum was added after the glycerol shock. After 2 days, the cells were split 1:5 and allowed to grow for 24 h prior to the addition of the neomycin analogue G418 (Gibco; 250–500 active µg/ml, depending on the batch). The medium was changed every 3 days. After 2–3 weeks the surviving cells were harvested and pooled for CAT assay.

Cells were continuously propagated in medium with G418. When needed, the cells were treated for 24 h with the relevant hormones, in the absence of G418, as described in the legends to the figures.

Assay of CAT activity

Cell extracts were prepared and CAT assays were performed according to the technique of Gorman et al. [33]. CAT assays were performed on 150–300 μ g of protein at 37 °C for 1–2 h. The proteins were assayed according to the method of Bradford [34].

RESULTS

The effects of phorbol esters on cAspAT activity were investigated in Fao cells. As shown in Figure 1, increasing concentrations of PdBu had no significant effect on basal cAspAT activity. Dexamethasone, a synthetic glucocorticoid agonist, induced cAspAT activity 9-fold at 0.1 μ M, as expected [6]. This effect was partially inhibited by the phorbol esters in a dose-dependent manner. Maximal inhibition (40%) was attained at a concentration of 0.3 μ M PdBu (Figure 1).

The effect of PdBu described above was very similar to the inhibition by insulin of the glucocorticoid mediated induction of cAspAT [6]. Neither insulin nor PdBu modified basal cAspAT activity (Figure 2a). However, both inhibited the stimulation of enzyme activity by dexamethasone, approximately to the same extent (40–50%). When both compounds were added together, 70% inhibition was attained. In no case was the inhibition complete.

An insulinomimetic agent, vanadate, was also tested and was found to inhibit dexamethasone-induced cAspAT activity (Figure 2b), confirming the general properties of this compound [35,36]. In the experiment shown in Figure 2(b), the effects of PdBu and vanadate were similar (40% inhibition) and the addition of both compounds resulted in an additive inhibitory effect on the induced

Figure 1 Dose-response curve of the effect of phorbol esters on cAspAT activity in the presence or in the absence of dexamethasone

Fao cells were exposed for 24 h to phorbol esters in the presence (■) or absence (●) of 0.1 μ M dexamethasone, and the cAspAT activity was determined. The results are expressed as the means $\pm$ S.E.M. of six independent determinations. The cAspAT activities of the dexamethasone-treated and the dexamethasone-plus-phorbol-ester-treated cells were compared using Student's *t* test (**P* < 0.05).

Figure 2 Effects of phorbol esters, insulin and vanadate on cAspAT activity in Fao cells

(a) Cells were untreated (C), or treated with dexamethasone (0.1 μ M) (D), PdBu (0.6 μ M) (P) or insulin (0.1 μ M) (I), alone or in combination, for 24 h. (b) Cells were untreated (C) or treated with dexamethasone (0.1 μ M) (D), PdBu (0.3 μ M) (P) or vanadate (0.5 mM) (V), alone or in combination. (c) Fao cells, not preincubated (■) or preincubated (▨) with 0.6 μ M PdBu for 18 h, were either untreated or treated for 24 h with dexamethasone (0.1 μ M) (D), alone or in combination with PdBu (0.3 μ M) (D+P) or insulin (10 nM) (D+I). Each result is the mean $\pm$ S.E.M. of 7–9 (a), 4 (b) or 5 (c) independent determinations. The differences between cAspAT activity in C and D, D and D+P, D and D+I, D and D+P+I, D and D+V, and D and D+P+V were analysed statistically using Student's *t* test (a and c) or the Mann-Whitney *U* test (b): ***P* < 0.001; **P* < 0.05.

cAspAT activity. It is noteworthy that the inhibitory effects of insulin and vanadate were not additive (results not shown).

In order to investigate the role of PKC in the inhibitory effect of PdBu, cells were preincubated with a high concentration of PdBu (0.6 μ M) for 18 h, which results in down-regulation of PKC [12,37]. Basal levels and the extent of induction by dexamethasone were not modified (Figure 2c). The inhibitory effect of PdBu was abolished in preincubated cells, suggesting that PKC was critical for PdBu action. Interestingly, insulin inhibited cAspAT induction by dexamethasone in both control and pretreated cells (Figure 2c). These results show that the effects of insulin and PdBu can be clearly distinguished, and that insulin was still active despite the down-regulation of PKC. It should be noted, however, that some PKC isoenzymes are not down-regulated by prolonged treatment by phorbol ester [38]. Therefore our data do not eliminate the possibility that insulin action may be mediated by a PKC isoenzyme. They simply

Figure 3 Effect of a 3 h treatment with dexamethasone, phorbol esters and insulin on the amounts of cAspAT, mAspAT and TAT mRNAs

Fao cells were untreated (C), or treated with 0.1 μ M dexamethasone (D) or 0.6 μ M PdBu (P), alone or in combination, for 3 h. Northern blot analysis was performed three times, each giving similar results. The bands on the Northern blots were quantified by scanning densitometry.

suggest that, if this were to be the case, PdBu and insulin would activate different PKC isoenzymes.

The molecular mechanism by which PdBu prevented the increase in cAspAT activity by dexamethasone was investigated by Northern blot analysis (Figure 3). Two mRNA species are labelled by a cAspAT probe, one major form at 2.1 kb and one minor form at 1.8 kb. We have already shown that dexamethasone induces cAspAT mRNAs and that insulin partially prevents the glucocorticoid effect [6]. In Figure 3 we show that PdBu had an effect similar to that of insulin. It did not modify the basal levels of the cAspAT mRNAs. However, it partially inhibited the induction by dexamethasone of these mRNAs (approx. 60%). The mRNAs of mAspAT were not modified by PdBu treatment (Figure 3), in agreement with the absence of regulation of this enzyme activity by PdBu (results not shown). TAT mRNA was also regulated by dexamethasone. However, in the presence of dexamethasone, PdBu had no effect on TAT mRNA. We conclude that the inhibitory effect of PdBu on cAspAT induction by glucocorticoids is gene-specific and does not result from a general inhibition of glucocorticoid action.

Figure 4 Nuclear run-on transcription assay

Dexamethasone (0.1 μ M) (D) was added to Fao cells alone or in combination with PdBu (0.6 μ M) (P) or insulin (0.1 μ M) (I). Nuclei were isolated 2 h after exposure of the Fao cells to these drugs and a nuclear run-on transcription assay was performed. The labelled RNA transcripts were hybridized to Hybond nylon filters containing 10 μ g of the linearized vector (negative control; lane 1), the tubulin cDNA plasmid (lane 2), the 28 S ribosomal cDNA plasmid (lane 3) (positive controls) or the cAspAT cDNA plasmid (lane 4), and quantification of the signal was carried out by densitometric analysis. Run on experiments were performed twice, each giving similar results.

In order to determine whether the inhibition of the cAspAT mRNAs mediated by PdBu was due to a modification in the transcription of the cAspAT gene, nuclear run-on assays were performed. Nuclei were isolated from Fao cells that were untreated or treated with dexamethasone alone or in combination with PdBu or insulin. Basal transcription of the cAspAT gene was low, yielding a signal just above the background (Figure 4). Treatment with dexamethasone alone increased the transcription rate approx. 6-fold. Addition of PdBu or insulin decreased the stimulation by 40–60%. The specificity of this regulation was confirmed by the constitutive transcription of the tubulin gene. These data show that the regulation of cAspAT as described here occurs at the transcriptional level.

Transcriptional regulation usually occurs at the promoter level. We have cloned the promoter of the rat cAspAT gene and sequenced 4 kb of upstream regulatory region ([30]; M. Aggerbeck, unpublished work). A fragment of this promoter (–2405/+26) (+1 corresponds to the translation initiation site; see [30]) was inserted upstream of the CAT gene and transfected into the Fao cells. Two types of transfection experiment were carried out. Transient transfections, in which the bulk of added DNA is not integrated into the genomic DNA, yielded poorly reproducible data. Permanently transfected cells were obtained by co-transfecting a neomycin resistance gene followed by selection with the compound G 418. A pool of resistant clones was propagated and was used for studies on the regulation of the cAspAT promoter. These cells were incubated for 24 h with 0.6 μ M PdBu, or 10 nM insulin, in the presence or in the absence of dexamethasone (Table 1).

The promoter activity carried by the p–2405/+26 CAT construct was not regulated by PdBu or insulin alone (results not shown). However, PdBu and insulin inhibited the stimulation by glucocorticoid of the promoter activity (50% inhibition; Table 1). In five independent transfections performed with three different plasmid preparations, inhibitory effects of PdBu and insulin were observed. The data suggest that, in the regulation of cAspAT activity, PdBu exerts its effects upon the 2.4 kb fragment of the cAspAT gene promoter, thus confirming the results obtained in the run-on experiments.

Table 1 Regulation of the cAspAT gene promoter by dexamethasone, phorbol esters and insulin

Five independent stable transfection experiments were performed using three different plasmid preparations (experiments 1–3 were done with the same plasmid preparation). Each value is the mean of duplicate determinations. In these experiments cells were either treated or not for 24 h with 0.1 μ M dexamethasone alone or in combination with 0.6 μ M PdBu or 10 nM insulin.

Experiment	Stimulation by dexamethasone (fold)	Inhibition by PdBu (%)	Inhibition by insulin (%)
1	3	60	52
2	12	50	49
3	13	35	28
4	5	29	34
5	10	41	24

DISCUSSION

The regulation by phorbol esters of cAspAT activity and gene expression was analysed in the Fao rat hepatoma cell line. We used these cells for several reasons. First, they are differentiated hepatoma cells which have conserved several metabolic functions of the adult rat liver, in particular gluconeogenesis [22]. Secondly, phorbol esters have been shown to modify other enzymic activities in these cells as well as in the H₄IIEC₃ cell line from which they are derived [12,19]. Finally, we have analysed the regulation of cAspAT by other hormones or effectors in these cells. The Fao cells are thus a good model for the analysis of the complex regulation of this housekeeping gene.

We have already reported that glucocorticoids induce cAspAT gene expression in the Fao cells [6]. This induction is potentiated by cyclic AMP and inhibited by insulin. Here we show that the effect of phorbol esters on cAspAT activity is similar to that of insulin and opposite to that of cyclic AMP. Therefore, activators of protein kinase A (PKA) and PKC have opposite effects on cAspAT expression. The physiological relevance of regulation of cAspAT is straightforward by PKA or cyclic AMP, since cAspAT participates in gluconeogenesis [2,39]. Other gluconeogenic enzymes such as PEPCK are also induced by cyclic AMP [40]. The physiological significance of the negative effect of PKC activators on cAspAT expression is less clear, however. It should be noted that a similar inhibitory effect has already been reported for PEPCK [13,19], and is therefore a property of at least two enzymes of the gluconeogenic pathway. Diacylglycerol, the endogenous activator of PKC, is produced following the stimulation of phospholipase C [41] or phospholipase D [42] by several hormones and growth factors. Some of these hormones, such as vasopressin, adrenaline and angiotensin, are known to modify glucose metabolism, but they act mostly by activating glycogenolysis [43]. This activation is mediated by the increase in cytosolic calcium concentration and the stimulation of PKC activity [44,45]. In the case of glycogenolysis, the effect of these hormones is similar to that of cyclic AMP and different from that of insulin.

The inhibition of cAspAT and PEPCK by phorbol esters displays some differences. In the case of cAspAT, phorbol esters inhibit the induction of the enzyme by glucocorticoids, rather than inhibiting the basal activity itself as in the case of PEPCK [13]. However, this is a general property of cAspAT regulation; both cyclic AMP and insulin modulate the stimulation of cAspAT

by glucocorticoids but neither affect the basal level of the enzyme [4]. Furthermore, the induction of cAspAT by glucocorticoids is tissue-specific, whereas the basal expression is ubiquitous. The data on regulation by phorbol esters is in keeping with those observations and confirm that cAspAT gene expression is regulated by several factors, but only in the presence of glucocorticoids [6].

A central question in insulin action concerns the similarity of this action with that of phorbol esters [9]. There have been several reports on the insulinomimetic effects of phorbol esters, among them activation of ornithine decarboxylase [12], stimulation of *c-fos* proto-oncogene expression [11], increased uptake of glucose and amino acids [18] and inhibition of PEPCK [13] and cAspAT (the present study). This similarity suggests that insulin may act by activating PKC. Indeed, there have been some reports on an increase in PKC activity following insulin treatment [9,46]. Insulin has been shown to activate one isoenzyme of PKC, i.e. PKC- ϵ [47]. However, several observations argue against such mechanisms. (i) In some cases, insulin and phorbol esters produce opposite effects, as reported for TAT [48]. (ii) When PKC is down-regulated, several effects of insulin are conserved, whereas the effects of phorbol esters are clearly decreased [12,19]; this is the case for cAspAT as reported here. Many of these experiments have been undertaken in hepatoma cells derived from H4IIEC3 cell lines, including Fao cells. Desensitization of PKC was obtained by preincubation with high concentrations of phorbol esters. In other experiments, inhibitors of PKC have been used and shown not to alter the effects of insulin [19]. (iii) The activation of PKC by insulin has been re-evaluated by transfection with vectors coding for the MARCKS (myristoylated alanine-rich C kinase substrate) protein, which is an excellent substrate for PKC [49]. Of five cell lines studied, insulin induced phosphorylation of the MARCKS protein in only one of them, and with a much lower efficiency than that occurring with phorbol esters. The cell line in question expresses the insulin receptor at a very high level. Interestingly, insulin had no effect in H4IIEC3 hepatoma cells, which also express insulin receptors. These data tend to suggest that the effect of insulin on PKC is not crucial for its activity. This conclusion is in agreement with our data on cAspAT regulation.

In order to account for the similarity in the effects of insulin and phorbol esters, it has been suggested that the events elicited by these effectors converge at some level, probably downstream of PKC. This suggestion was strengthened by the isolation of an IRS (insulin-responsive sequence) element in the PEPCK promoter which accounts, at least partially, for the effects of insulin as well as those of phorbol esters [21]. As already mentioned, the signal transduction pathways of these effectors have been shown to be distinct in their initial steps. It is possible that a similar mechanism occurs in the case of cAspAT promoter regulation. A sequence identical to the IRS element is present in the cAspAT promoter. However, mutation of this sequence did not abolish the insulin or phorbol ester regulation of cAspAT (M. Aggerbeck and S. Feilleux-Duché, unpublished work). The identification of the element that is responsive to one of these effectors should enable us to determine whether the insulin and phorbol ester pathways also converge at the level of the cAspAT promoter, yielding a similar regulation. It is possible that this element may be indistinguishable from the glucocorticoid response elements mediating the stimulation of cAspAT by glucocorticoids. Indeed, neither phorbol esters nor insulin modify basal promoter activity, but rather inhibit its induction by dexamethasone. It has been reported that the glucocorticoid receptor and the phorbol 12-myristate 13-acetate (PMA)-sensitive AP1-complex proteins interact even in the absence of DNA [50]. This interaction results

in inhibition by glucocorticoids of PMA-mediated gene stimulation, and conversely may also account for the inhibition by PMA of glucocorticoid-mediated gene induction [51]. Further studies will help determine at which level phorbol esters and insulin inhibit the stimulation of cAspAT gene transcription by glucocorticoids.

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