Pretranslational regulation of tyrosine aminotransferase and phosphoenolpyruvate carboxykinase (GTP) synthesis by glucagon and dexamethasone in adult rat hepatocytes

Patrick B. IYNEDJIAN,* Patrick AUBERGER, Yves GUIGOZ† and Alphonse LE CAM‡
Institut National de la Santé et de la Recherche Médicale, Unité de Recherches sur les Hormones Polypeptidiques et la Physiopathologie Endocrinienne (I.N.S.E.R.M. U 145), Faculté de Médecine, Avenue de Vallombrose, 06034 Nice Cédex, France

(Received 29 June 1984/Accepted 4 September 1984)

The regulation of synthesis of the gluconeogenic cytosolic enzyme phosphoenolpyruvate carboxykinase (PEPCK) and of tyrosine aminotransferase (TAT) by glucagon and glucocorticoid hormones was studied in hepatocytes maintained in suspension culture for 7 h. Specific antibodies were used to measure relative rates of enzyme synthesis after pulse-labelling of the cells with [³H]leucine or [³⁵S]methionine. Concomitantly, amounts of mRNA were quantified after translation *in vitro* in a reticulocyte lysate and specific immunoprecipitation of the proteins. Glucagon stimulated the rate of synthesis of PEPCK by 4–6-fold and that of TAT by 6–8-fold in 2 h. In contrast, dexamethasone had little effect on PEPCK synthesis, whereas it increased TAT synthesis by 5–9-fold. When used in combination, the two hormones displayed additive effects on TAT synthesis, whereas the glucocorticoid hormone strongly potentiated stimulation of PEPCK synthesis by glucagon. In every instance, changes in rates of synthesis of the two enzymes were totally accounted for by increases in amounts of the corresponding functional mRNA, suggesting a pretranslational site of action for both glucagon and dexamethasone.

Tyrosine aminotransferase (EC 2.6.1.5) and the cytosolic form of phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) are well-known inducible enzymes of the mammalian liver. Both have been under intensive investigation as model systems to understand the regulation of specific gene expression in the liver, especially as regards the mechanisms of action of cyclic AMP and the glucocorticoids. In the rat, glucagon causes a stimulation of the synthesis of tyrosine aminotransferase and phosphoenolpyruvate carboxykinase, resulting in increased enzyme activities (Wicks *et al.*, 1969; Hanson *et al.*, 1973). This effect is mimicked by injections of dibutyryl cyclic AMP and has been shown to result from an

accumulation of the corresponding mRNAs, as determined by translation as well as by hybridization assays (Iynedjian & Hanson, 1977; Ernest & Feigelson, 1978; Noguchi *et al.*, 1978; Beale *et al.*, 1982; Cimbala *et al.*, 1982). Tyrosine aminotransferase mRNA and its relative rate of synthesis are also rapidly increased after a single administration of cortisol (Roewekamp *et al.*, 1976; Diesterhaft *et al.*, 1980). The glucocorticoids, however, appear to be less effective inducers of hepatic phosphoenolpyruvate carboxykinase *in vivo*. A moderate stimulation of phosphoenolpyruvate carboxykinase synthesis has been noted in the diabetic, but not in the normal, animal after glucocorticoid administration (Gunn *et al.*, 1975).

Since the synthesis rates of tyrosine aminotransferase and phosphoenolpyruvate carboxykinase are controlled *in vivo* by cyclic AMP, the glucocorticoids, as well as several additional effectors (for reviews, see Tilghman *et al.*, 1976; Granner & Hargrove, 1983), cell-culture systems are needed to investigate the mechanisms of action of individual hormones under well-defined conditions. Liver

Abbreviation used: SDS, sodium dodecyl sulphate.

* Present address: Institut de Biochimie Clinique, Université de Genève, Sentier de la Roseaie, 1211 Genève, Switzerland.

† Present address: Nestlé Research Department, 1814 La Tour-de-Peilz, Switzerland.

‡ To whom correspondence and reprint requests should be addressed.

cells in suspension culture were shown to respond to glucagon or cyclic AMP by a rapid induction of phosphoenolpyruvate carboxykinase synthesis, an effect markedly enhanced in the presence of dexamethasone (Salavert & Iynedjian, 1982). This prompted us to undertake similar studies on the regulation of tyrosine aminotransferase, especially in view of conflicting reports on the inducibility of this enzyme in isolated hepatocytes (Ernest *et al.*, 1977; Gurr & Potter, 1980; Auberger *et al.*, 1983). The present paper describes the effects of glucagon and dexamethasone on the amounts of mRNA species coding for tyrosine aminotransferase and phosphoenolpyruvate carboxykinase and on the relative rates of synthesis of the two enzymes. The specific issues that we have addressed pertain to a possible translational control of hepatic enzyme synthesis by cyclic AMP (Wicks, 1974) and to the interaction of the glucocorticoids with the cyclic AMP system in the regulation of gene expression.

Materials and methods

Materials

Crystallized highly purified pig glucagon was generously given by Dr. Jorgen Schlichtkrull, Novo Research Institute, Copenhagen, Denmark. The mRNA-dependent rabbit reticulocyte-lysate translation system was bought as a kit from New England Nuclear. Radioactive L-[³⁵S]methionine (sp. radioactivity 1160 Ci/mmol) and L-[3,4,5-³H]leucine (sp. radioactivity 110–140 Ci/mmol) were from the same supplier. Triton X-405 was from Fluka; protein A-Sepharose 6 MB and CL4B were from Pharmacia. *M_r* markers for gel electrophoresis were [¹⁴C]methylated proteins from Amersham. Enhancing solution for fluorography was En³Hance from New England Nuclear.

Purification of tyrosine aminotransferase and production of antibodies

Tyrosine aminotransferase was purified as described by Hargrove *et al.* (1980). Before its utilization for rabbit immunization, the purified protein was submitted to electrophoresis in a 10% polyacrylamide gel containing SDS, in order to separate the subunit of tyrosine aminotransferase form I from proteolytic fragments which formed during storage of the enzyme. After electrophoresis, the gel was soaked for 20 min in 4M-sodium acetate and viewed under fluorescent light to localize protein bands (Higgins & Dahmus, 1979). The band containing the intact enzyme subunit was cut out from the gel, crushed in phosphate-buffered saline and the gel paste was mixed with an equal volume of complete Freund's adjuvant. White rabbits were injected with approx. 0.2 mg of protein/animal at several intradermal sites. Intra-

dermal booster injections were administered 3 weeks later, followed by two subsequent intramuscular injections at 3-week intervals. Immune sera were obtained 6 days after the last booster shot.

Liver-cell isolation

Hepatocytes were isolated by perfusion of the liver with collagenase essentially as described by Seglen (1976). Before the experiments, male Wistar rats weighing about 160 g were starved for 20 h and subsequently re-fed with 10 g of glucose/kg body wt. by gavage. Surgery for liver perfusion was performed under pentobarbital anaesthesia 2 h after re-feeding. The liver was initially flushed *in situ* with 150 ml of 10 mM-Hepes [4-(2-hydroxy-ethyl)-1-piperazine-ethanesulphonic acid] buffer, pH 7.6, containing heparin (100 units/ml) and EGTA (0.6 mM), followed by 150 ml of Hepes buffer alone. Collagenase was then perfused for 6 min at 50 ml/min in a recirculating circuit at a concentration of 0.5 mg/ml in a solution containing 10 mM-CaCl₂. All buffers were equilibrated with CO₂/O₂ (1:19) and perfused at 37°C. At the end of the perfusion, the cells were dissociated and washed as described by Salavert & Iynedjian (1982). Cells were cultured in suspension for 7–8 h in 250 ml Erlenmeyer flasks containing 60 ml of a medium defined previously (Salavert & Iynedjian, 1982), to which was added 2 mM-sodium pyruvate. The cell density was approx. 10⁶ cells/ml of medium. Viability estimated by Trypan Blue exclusion was better than 90% at the end of the experiments.

Pulse-labelling of cells

Cells were withdrawn from incubation flasks, washed once in amino acid-free incubation medium and transferred to amino acid free-medium containing 250 μCi of [³⁵S]methionine/ml or 400 μCi of [³H]leucine/ml. Routinely, 750 × 10³ cells were labelled in a volume of 0.3 ml. Labelling was terminated after 15 min by centrifuging the cells (200 g for 2 min) and washing in ice-cold non-radioactive medium. Thereafter, cells were resuspended in 0.17 ml of a solution containing 25 mM-potassium phosphate, pH 7.8, 0.12 M-KCl, 0.1 mM-pyridoxal phosphate, 1 mM-dithiothreitol, 1 mM-α-oxoglutaric acid (homogenization buffer), supplemented with 0.1 mM-phenylmethanesulphonyl fluoride. Cells were disrupted by three cycles of freezing-thawing.

Indirect immunoprecipitation of tyrosine aminotransferase and phosphoenolpyruvate carboxykinase

Cellular homogenates were centrifuged at 100 000 *g*_{max} for 15 min at room temperature. The particle-free supernatant was withdrawn and pre-adsorbed with protein A-Sepharose CL 4B for 2 h at 4°C, by using 0.1 ml of packed beads for 0.15 ml

of high-speed supernatant. After pelleting of the beads by centrifugation (800g, 5min), the supernatant was removed and the beads were washed with 2×0.125 ml of cold homogenization buffer. All the supernatants were then pooled (vol. 0.4 ml) and divided into two samples for immune precipitation, 0.260 ml for tyrosine aminotransferase and 0.130 ml for phosphoenolpyruvate carboxykinase. Antibodies in amounts sufficient to neutralize about twice the amounts of enzyme present in the cell extract were added together with Triton X-405 (final concn. 0.6%). The mixtures were incubated at 37°C for 1 h. An excess of protein A-Sepharose CL 4B was then added, and incubation was continued with end-over-end agitation for 3 h at 4°C. Protein A-Sepharose was pelleted by centrifugation (800g, 5min) and the supernatant was removed. The beads were washed with 5×0.6 ml of homogenization buffer containing 5 mM-methionine or 5 mM-leucine and 0.6% Triton X-405. After two more washes with phosphate-buffered saline, electrophoresis sample buffer containing SDS and β -mercaptoethanol was added to the beads, and immune complexes were eluted and dissociated by incubation at 100°C for 10 min. The supernatants obtained after pelleting of the beads were loaded on SDS/polyacrylamide slab gels prepared as described by Laemmli (1970) or on cylindrical gels prepared as described by Weber & Osborn (1969). After electrophoresis, slab gels were soaked in En³Hance, dried and used for fluorography on Kodak X-Omat AR films at -70°C. Cylindrical gels were sliced for liquid-scintillation counting. To quantify the rate of total protein synthesis, samples of high-speed supernatant from cellular homogenates were precipitated with 10% (w/v) trichloroacetic acid, washed several times in trichloroacetic acid and dissolved in 0.1 M-NaOH for liquid-scintillation counting.

Isolation and translation of RNA

Liver cell RNA was isolated by the method of Chirgwin *et al.* (1979), exactly as described previously (Salavert & Iynedjian, 1982). Total cellular RNA was translated in a commercial mRNA-dependent rabbit reticulocyte-lysate system, under conditions specified by the manufacturer. Routinely, the reaction volume was 75 μ l containing 30 μ l of lysate and 15 μ g of RNA. Incubation was at 36°C for 60 min. Thereafter, reaction mixtures were diluted by adding 90 μ l of homogenization buffer and centrifuged at 100 000 g_{max} for 20 min at room temperature. The high-speed supernatant was pre-adsorbed for 30 min at 4°C with 30 μ l of packed protein A-Sepharose 6 MB and then used sequentially for indirect immunoprecipitations of newly synthesized tyrosine aminotransferase and phospho-

enolpyruvate carboxykinase. Anti-(tyrosine aminotransferase) antiserum was added first and the mixture was incubated for 1 h at 37°C and 3 h at 4°C. The mixture was transferred on to 45 μ l of packed protein A-Sepharose 6 MB and the incubation continued for 45 min at 37°C with frequent mixing. The affinity beads were collected by centrifugation (800g, 5min), and subsequently washed and eluted in electrophoresis sample buffer as described above. The supernatant was utilized for indirect immunoprecipitation of phosphoenolpyruvate carboxykinase by the same procedure. Products eluted from the protein A-Sepharose adsorbent were analysed by SDS/polyacrylamide-gel electrophoresis as described above. In control experiments, the supernatant left after immunoprecipitation of the two enzymes was analysed by high-resolution two-dimensional gel electrophoresis as described by O'Farrell (1975) and found to be virtually totally depleted of tyrosine aminotransferase and phosphoenolpyruvate carboxykinase translation products, demonstrating that immunoprecipitation was quantitative. Samples of high-speed supernatant from translation mixtures were used for analysis of the overall pattern of products by one- or two-dimensional gel electrophoresis.

Results

Glucagon and dexamethasone effects on enzyme rates of synthesis

The rates of synthesis of tyrosine aminotransferase and phosphoenolpyruvate carboxykinase were measured by indirect immunoprecipitation of the enzymes from cell extracts prepared after pulse-labelling of hepatocytes with [³⁵S]methionine. The antibodies to phosphoenolpyruvate carboxykinase have been characterized previously (Iynedjian & Jacot, 1980; Salavert & Iynedjian, 1982). The specificity of the rabbit antiserum against tyrosine aminotransferase is demonstrated in Fig. 1. Total labelled cytosol proteins (tracks 1 and 2) and immuno-adsorbed polypeptides (tracks 3 and 4) from control cells and cells challenged with glucagon are displayed after electrophoresis in a SDS/polyacrylamide slab gel. The antibodies recognize a major polypeptide with apparent M_r 55 000 and a minor polypeptide with apparent M_r 51 000. These polypeptides correspond respectively to the subunits of forms I and III of tyrosine aminotransferase, as defined by Hargrove *et al.* (1980). These investigators showed that subunit form III is derived from form I by proteolytic cleavage during tissue homogenization and that proteolysis can be largely prevented by using the homogenization buffer described in the Materials and methods section. Clearly, radioactive amino

Fig. 1. Fluorogram showing total cytosol proteins (tracks 1 and 2) and immunoprecipitable tyrosine aminotransferase (tracks 3 and 4) synthesized by hepatocytes in suspension culture

Cells were pulse-labelled with [^{35}S]methionine for 15 min after 7 h of incubation in basal medium (tracks 1 and 3) or 5 h in basal medium, followed by 2 h in medium supplemented with $0.4\text{ }\mu\text{M}$ -glucagon (tracks 2 and 4). Procedures for cytosol preparation and indirect immunoprecipitation of tyrosine aminotransferase are described in the Materials and methods section. Electrophoresis was in a 10%-polyacrylamide gel containing SDS. M_r markers were: phosphorylase *b* (92500), serum albumin (69000), ovalbumin (46000), carbonic anhydrase (30000), lysozyme (14300). I and III designate the two different forms of the enzyme (see the text); OR, origin.

acid incorporation into both forms of tyrosine aminotransferase is increased in glucagon-treated cells relative to control cells (cf. tracks 3 and 4), whereas incorporation into most cytosolic proteins appears unaltered (tracks 1 and 2).

In the following experiments, cells were preincubated in hormone-free medium for 5 h before stimulation with glucagon ($0.4\text{ }\mu\text{M}$), dexamethasone ($0.1\text{ }\mu\text{M}$) or both hormones simultaneously. Incubation was continued for 2 h in the presence of the hormones, and the cells were subsequently pulse-labelled with [^3H]leucine for 15 min. Leucine

Table 1. Induction of tyrosine aminotransferase (TAT) and phosphoenolpyruvate carboxykinase (PEPCK) synthesis by glucagon and dexamethasone. Cells were preincubated for 5 h in hormone-free medium before hormone additions. The time at which additions were made is zero time. Pulse-labelling of cells and measurements of [^3H]leucine incorporation into total and specific proteins were performed as detailed in the text and the Materials and methods section. Incorporation values for tyrosine aminotransferase include incorporation in both subunit types. Induction (fold) is the ratio of relative rate of enzyme synthesis under induced condition to relative rate of synthesis at zero time. Data are means $\pm$ s.e.m. of three distinct experiments, except for 'Induction', for which the range of values is indicated.

Incubation conditions	[³ H]Leucine incorporation into:			Relative rate of synthesis (p.p.m.)				Induction (fold)	
	Total cytosol proteins (c.p.m./750 cells)	TAT (c.p.m./750 × 10 ³ cells)	PEPCK (c.p.m./750 × 10 ³ cells)	TAT		PEPCK		TAT	PEPCK
				(c.p.m./750 × 10 ³ cells)		(c.p.m./750 × 10 ³ cells)			
Zero time	536 ± 15	186 ± 47	627 ± 175	344 ± 83	1177 ± 343	1	1	1	1
No hormone (2h)	819 ± 101	335 ± 75	1261 ± 394	415 ± 95	1491 ± 336	—	—	—	—
Glucagon, 0.4 μM (2h)	704 ± 92	2509 ± 270	4585 ± 1114	3667 ± 656	6399 ± 977	5.5–8	5.5–8	4.5–6	4.5–6
Dexamethasone, 0.1 μM (2h)	619 ± 78	2063 ± 324	1173 ± 224	3314 ± 213	1873 ± 215	5–9	5–9	1–1.8	1–1.8
Glucagon + dexamethasone (2h)	526 ± 73	3641 ± 605	8795 ± 1280	6895 ± 450	16710 ± 92	10–20	10–20	11–14	11–14

incorporation into total cytosol proteins was measured by trichloroacetic acid precipitation. Incorporation into both enzymes was measured immunochemically as shown in Fig. 1 for tyrosine aminotransferase, except that immunoadsorbed proteins were electrophoresed in cylindrical SDS/polyacrylamide gels and appropriate slices of the gels were cut for radioactivity measurements. Data from these induction experiments are presented in Table 1. The relative rate of synthesis of tyrosine aminotransferase was increased 6–8-fold 2 h after addition of glucagon. In the same cells, phosphoenolpyruvate carboxykinase synthesis was induced 4–6-fold. Previous work has shown that $0.4 \mu\text{M}$ -glucagon is a maximal concentration for phosphoenolpyruvate carboxykinase induction and that enzyme synthesis virtually reaches a plateau 2 h after hormone addition (Salavert & Iynedjian, 1982). Dexamethasone caused a 5–9-fold increase in the relative rate of tyrosine aminotransferase synthesis, compared with a less than 2-fold stimulation of phosphoenolpyruvate carboxykinase synthesis. The effects of glucagon and dexamethasone on tyrosine aminotransferase synthesis were additive. Significantly, however, the stimulation of phosphoenolpyruvate carboxykinase synthesis by glucagon and dexamethasone together was supra-additive, reflecting a synergism between the two inducers.

Glucagon and dexamethasone effects on enzyme mRNA amounts

Tyrosine aminotransferase and phosphoenolpyruvate carboxykinase mRNA species were assayed translationally in a mRNA-dependent reticulocyte-lysate protein-synthesis system. Translation products encoded by total cellular RNA from hepatocytes induced with glucagon and dexamethasone were analysed by high-resolution two-dimensional gel electrophoresis. Fig. 2 shows the overall pattern of newly synthesized polypeptides (a) and the product reacting with anti-tyrosine aminotransferase antibodies (b). The immunoreactive translation product migrates with an apparent pI of 5.5–5.9 and an apparent M_r of 55 000. These values are in general agreement with published data (Hargrove *et al.*, 1980; Hargrove & Granner, 1981), although charge heterogeneity is more pronounced in the present experiment. This experiment confirmed the specificity of the anti-(tyrosine aminotransferase) antiserum and allowed us to identify the primary translation product of tyrosine aminotransferase mRNA in high-resolution two-dimensional gels containing total translation products (downward arrow, Fig. 2a). The translation product of phosphoenolpyruvate carboxykinase mRNA (upward arrow, Fig. 2a) has been similarly identified (Iynedjian & Jacot, 1981).

Fig. 2. Fluorogram showing total released translation products (a) and immunoprecipitable tyrosine aminotransferase (b) synthesized by rabbit reticulocyte lysate. Template RNA was total cellular RNA isolated from hepatocytes induced with glucagon plus dexamethasone. Translation was performed in the presence of [^{35}S]methionine. High-resolution two-dimensional gel electrophoresis was by the method of O'Farrell (1975), with a 10%-polyacrylamide gel containing SDS in the second dimension. The downward arrow indicates tyrosine aminotransferase, and the upward arrow phosphoenolpyruvate carboxykinase. Numbers at the top indicate pH values along the gradient.

A comparison of the intensity of the two spots indicates that phosphoenolpyruvate carboxykinase is not synthesized more actively than tyrosine aminotransferase in the reticulocyte-lysate translation system. In the liver cell, on the contrary, the relative rate of synthesis of phosphoenolpyruvate carboxykinase synthesis is about twice that of tyrosine aminotransferase (see Table 1). It appears therefore that phosphoenolpyruvate carboxykinase mRNA is translated rather inefficiently in the heterologous protein-synthesis system, presumably because of its large size or secondary structure.

Total RNA was extracted from cells induced with glucagon, dexamethasone or both hormones together and translated in the reticulocyte lysate. Immunoabsorbed tyrosine aminotransferase and phosphoenolpyruvate carboxykinase were electrophoresed alongside with total released translation

Fig. 3. Fluorogram showing the effects of glucagon and dexamethasone on translatable mRNA species coding for tyrosine aminotransferase (TAT) and phosphoenolpyruvate carboxykinase (PEPCK)

Total cellular RNA from hepatocytes was translated by the mRNA-dependent rabbit reticulocyte lysate in the presence of [35 S]methionine. Electrophoresis of the products was performed in a 10%-polyacrylamide gel containing SDS: tracks 2–6, total released polypeptides; tracks 7–11, tyrosine aminotransferase immunoreactive products; tracks 12–16, phosphoenolpyruvate carboxykinase immunoreactive products. The RNA samples for translation were isolated from cells before hormonal stimulation (tracks 2, 7, 12), 2h after addition of glucagon (tracks 3, 8, 13), 2h after addition of dexamethasone (tracks 4, 9, 14), 2h after addition of glucagon plus dexamethasone (tracks 5, 10, 15) or after continued incubation in basal medium (tracks 6, 11, 16). One-quarter of the translation mixture was used for analysis of total polypeptides. The remainder was used sequentially for tyrosine aminotransferase and phosphoenolpyruvate carboxykinase immunoprecipitations. Track 1: total translation products in absence of exogenous RNA. M_r markers were as in Fig. 1.

products in SDS/polyacrylamide gels. Fluorograms of such gels are shown in Fig. 3. All RNA preparations tested directed the synthesis of a similar range of polypeptides (tracks 2–6). The presence of high- M_r products suggests that RNA degradation was not a problem in these experiments. Both glucagon and dexamethasone caused an increase in the amount of functional tyrosine aminotransferase mRNA, as evidenced by an increase in the synthesis of full-length immunoreactive tyrosine aminotransferase in the lysate system. The effects of the two hormones were comparable in magnitude and appeared roughly additive (Fig. 3, tracks 7–11). In contrast, the effect of glucagon on phosphoenolpyruvate carboxykinase mRNA was more pronounced than that of dexamethasone. Furthermore, the combination of glucagon and dexamethasone appeared to cause a supra-additive build-up of phosphoenolpyruvate carboxykinase

Table 2. Effects of glucagon and dexamethasone on amounts of functional tyrosine aminotransferase (TAT) and phosphoenolpyruvate carboxykinase (PEPCK) mRNA species and rates of enzyme synthesis

The mRNA assay was by translation in the mRNA-dependent rabbit reticulocyte lysate, with liver cell total RNA as template. Technical details on RNA isolation and translation are described in the Materials and methods section. Relative mRNA activities are the ratios of amino acid incorporation into specific enzyme to mRNA-directed incorporation into total released products. Increase (fold) in mRNA is the ratio of mRNA activity under induced condition to mRNA activity before hormone addition. Relative rates of enzyme synthesis *in vivo* were determined as in Table 1. Data are from a representative experiment.

Incubation conditions	mRNA-directed [35 S]methionine incorporation into:				Relative mRNA activity (p.p.m.)				Increase (fold) in mRNA				Induction (fold) of enzyme synthesis <i>in vivo</i>			
	Total polypeptides (c.p.m./141 nl)		TAT (c.p.m./141 μ l)		PEPCK (c.p.m./141 μ l)		TAT		PEPCK		TAT		PEPCK		TAT	
Zero time	1577		462		234		293		148		1		1		1	
No hormone (2h)	1625		415		199		256		123		—		—		—	
Glucagon (0.4 μ M) (2h)	1744		2427		1451		1391		832		4.8		5.6		6.7	
Dexamethasone (0.1 μ M) (2h)	1713		3213		511		1876		298		6.4		2.0		2.2	
Glucagon + dexamethasone (2h)	1561		6351		2634		4068		1687		13.9		11.4		14.6	

mRNA (Fig. 3, tracks 12–16). Smaller polypeptides are seen together with full-size enzymes in immunoadsorbed products. Some appear to be abortive translation products of the specific enzyme mRNA molecules, or proteolytic fragments arising after completion of translation, since their abundance is related to that of full-size enzyme chains. Others are minor contaminants owing to non-specific adsorption during the immunochemical isolation procedure.

In order to quantify tyrosine aminotransferase and phosphoenolpyruvate carboxykinase mRNA, immunoreactive translation products were submitted to electrophoresis on cylindrical SDS/polyacrylamide gels, and the amount of [35 S]methionine in full-size products was measured by liquid-scintillation counting of appropriate gel slices. The overall template activity of the various RNA preparations was estimated from the total [35 S]methionine incorporated in hot-trichloroacetic acid-precipitable material. Data from a representative experiment in which the rates of enzyme synthesis *in vivo* were also measured are presented in Table 2. Glucagon and dexamethasone caused 5- and 6-fold increases in functional tyrosine aminotransferase mRNA respectively; joint stimulation by the two hormones resulted in a 14-fold induction of the mRNA. In comparison, functional phosphoenolpyruvate carboxykinase mRNA was increased nearly 6-fold by glucagon, about 2-fold by dexamethasone and more than 11-fold by glucagon plus dexamethasone. It is important to note that the increases in specific mRNA molecules caused by glucagon as well as dexamethasone were of sufficient magnitude to explain the stimulations of tyrosine aminotransferase and phosphoenolpyruvate carboxykinase synthesis seen in intact cells. Similar results were obtained in two additional experiments of the same design.

Discussion

Rat liver cells incubated as described here respond to glucagon by a 6–8-fold induction of tyrosine aminotransferase synthesis in 2 h. In induced cells, the amount of functional tyrosine aminotransferase mRNA is increased to the same extent as the relative rate of enzyme synthesis. This argues for an effect of glucagon, presumably mediated by cyclic AMP, on the transcription, processing, transport or stability of tyrosine aminotransferase mRNA, rather than for a primary (and selective) effect on the translation machinery of the hepatocytes. Earlier studies *in vivo* led to a similar conclusion, based on an increase in tyrosine aminotransferase mRNA in rat liver after administration of dibutyryl cyclic AMP (Ernest *et al.*, 1977; Noguchi *et al.*, 1978; Scherer *et al.*, 1982).

Culpepper & Liu (1983) showed that 8-bromocyclic AMP induces a rapid accumulation of tyrosine aminotransferase mRNA in H-4 rat hepatoma cells. As in the present work, the increase in mRNA was adequate to explain the concomitant stimulation of enzyme synthesis. Together, these data provide firm evidence that the cyclic AMP-mediated stimulation of tyrosine aminotransferase synthesis in normal or tumoral liver cells is primarily due to a pretranslational mechanism. The results presented here also point to a pretranslational site of action for cyclic AMP in the regulation of phosphoenolpyruvate carboxykinase expression, in agreement with previous investigations in normal liver cells or H-4 hepatoma cells (Salavert & Iynedjian, 1982; Iynedjian & Salavert, 1983; Chrapkiewicz *et al.*, 1982). Indeed, Lamers *et al.* (1982) have reported direct evidence for an increase in the transcription rate of the cytosolic phosphoenolpyruvate carboxykinase gene in isolated nuclei from cyclic AMP-treated rats. It is tempting to speculate that the build-up of tyrosine aminotransferase mRNA reflects a similar transcriptional effect on the gene. Nevertheless, additional mechanisms, such as stabilization of the mRNA molecules, may also play a role in the cyclic AMP-dependent induction of the enzymes. Moreover, Noguchi *et al.* (1982) showed that the rate of tyrosine aminotransferase synthesis in rat liver could be maintained at the induced value by repeated injections of dibutyryl cyclic AMP, whereas amounts of tyrosine aminotransferase mRNA were returning to basal values. This suggested the possibility of a longer-term translational effect of cyclic AMP on the synthesis of tyrosine aminotransferase. This issue should be resolved by extended time-course experiments using cultured hepatocytes.

The synthesis of tyrosine aminotransferase is readily induced by dexamethasone in the present liver cell system, as a consequence of a comparable increase in cognate mRNA. A similar effect of the glucocorticoids occurs in HTC hepatoma cells and *in vivo* in rat liver (Roewekamp *et al.*, 1976; Olsen *et al.*, 1980; Scherer *et al.*, 1982). The glucocorticoid–receptor complex has been shown to bind with high affinity to specific DNA sequences in the promotor region of a glucocorticoid-inducible mammary-tumour-virus gene (Payvar *et al.*, 1981; Pfahl, 1982). Evidence indicates that binding of the hormone–receptor complex to these sites enhances the transcription of the gene concerned (Chandler *et al.*, 1983; Pfahl *et al.*, 1983). Such a mechanism may account for the induction of tyrosine aminotransferase.

The amount of mRNA coding for phosphoenolpyruvate carboxykinase was increased only slightly in cells incubated with dexamethasone.

Previously, Salavert & Iynedjian (1982) noted that phosphoenolpyruvate carboxykinase synthesis and mRNA amount in cultured liver cells were virtually unaffected by dexamethasone in the absence of glucagon. The weakness or absence of response to dexamethasone alone is intriguing, in view of the discovery of a potential glucocorticoid-regulatory DNA sequence upstream of the phosphoenolpyruvate carboxykinase gene (Yoo-Warren *et al.*, 1983). Perhaps the access of the hormone-receptor complex to the regulatory site(s) is hindered owing to structural features of phosphoenolpyruvate carboxykinase gene chromatin.

Tyrosine aminotransferase and phosphoenolpyruvate carboxykinase are typical examples of gene products placed under control of both polypeptide and steroid hormones. As shown here, glucagon and the glucocorticoids regulate the expression of the two enzymes primarily at the pretranslational level, that is by increasing the cellular contents of the specific mRNA species. Simultaneous induction by the two hormones results in an additive build-up of tyrosine aminotransferase mRNA. Under the same conditions, however, the increase in phosphoenolpyruvate carboxykinase mRNA is supra-additive, reflecting a synergism between glucagon and dexamethasone. The basis for this difference remains to be clarified.

We thank G. Le Cam for technical assistance and for preparing the illustrations, G. Visciano for art work and M. L. Capolongo and J. Duch for typing the manuscript. P. B. I. was the recipient of an INSERM fellowship for foreign investigators.

References

- Auberger, P., Samson, M. & Le Cam, A. (1983) *Biochem. J.* **214**, 679–685
- Beale, E. G., Hartley, J. L. & Granner, D. K. (1982) *J. Biol. Chem.* **257**, 2022–2028
- Chandler, V. L., Maler, B. A. & Yamamoto, K. R. (1983) *Cell* **33**, 489–499
- Chirgwin, J. M., Przybyla, A. E., McDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299
- Chrapkiewicz, N. B., Beale, E. G. & Granner, D. K. (1982) *J. Biol. Chem.* **257**, 14428–14432
- Cimbala, M. A., Lamers, W. H., Nelson, K., Monahan, J. E., Yoo-Warren, H. & Hanson, R. W. (1982) *J. Biol. Chem.* **257**, 7629–7636
- Culpepper, J. A. & Liu, A. Y. C. (1983) *J. Biol. Chem.* **258**, 13812–13819
- Diesterhaft, M., Noguchi, T. & Granner, D. K. (1980) *Eur. J. Biochem.* **108**, 357–365
- Ernest, M. J. & Feigelson, P. (1978) *J. Biol. Chem.* **253**, 319–322
- Ernest, M. J., Chen, C. L. & Feigelson, P. (1977) *J. Biol. Chem.* **252**, 6783–6791
- Granner, D. K. & Hargrove, J. L. (1983) *Mol. Cell. Biochem.* **53/54**, 113–128
- Gunn, J. M., Hanson, R. W., Meyuhos, O., Reshef, L. & Ballard, F. J. (1975) *Biochem. J.* **150**, 195–203
- Gurr, J. A. & Potter, V. R. (1980) *Ann. N.Y. Acad. Sci.* **349**, 59–66
- Hanson, R. W., Fisher, L., Ballard, F. J. & Reshef, L. (1973) *Enzyme* **15**, 97–110
- Hargrove, J. L. & Granner, D. K. (1981) *J. Biol. Chem.* **256**, 8012–8017
- Hargrove, J. L., Diesterhaft, M., Noguchi, T. & Granner, D. K. (1980) *J. Biol. Chem.* **255**, 71–78
- Higgins, R. C. & Dahmus, M. E. (1979) *Anal. Biochem.* **93**, 257–260
- Iynedjian, P. B. & Hanson, R. W. (1977) *J. Biol. Chem.* **252**, 655–662
- Iynedjian, P. B. & Jacot, M. M. (1980) *Eur. J. Biochem.* **111**, 89–98
- Iynedjian, P. B. & Jacot, M. M. (1981) *J. Biol. Chem.* **256**, 7068–7076
- Iynedjian, P. B. & Salavert, A. (1983) in *Isolation, Characterization and Use of Hepatocytes* (Harris, R. A. & Cornell, N. W., eds.), pp. 573–578, Elsevier Biomedical, New York, Amsterdam and Oxford
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Lamers, W. H., Hanson, R. W. & Meisner, H. M. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5137–5141
- Noguchi, T., Diesterhaft, M. & Granner, D. K. (1978) *J. Biol. Chem.* **253**, 1332–1335
- Noguchi, T., Diesterhaft, M. & Granner, D. K. (1982) *J. Biol. Chem.* **257**, 2386–2390
- O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021
- Olsen, P. S., Thompson, E. B. & Granner, D. K. (1980) *Biochemistry* **19**, 1705–1711
- Payvar, F., Wrange, O., Carlstedt-Duke, J., Okret, S., Gustafsson, J. A. & Yamamoto, K. R. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6628–6632
- Pfahl, M. (1982) *Cell* **31**, 475–482
- Pfahl, M., McGinnis, D., Hendricks, M., Groner, B. & Hynes, N. E. (1983) *Science* **222**, 1341–1343
- Roewekamp, W. G., Hofer, E. & Sekeris, C. E. (1976) *Eur. J. Biochem.* **70**, 259–268
- Salavert, A. & Iynedjian, P. B. (1982) *J. Biol. Chem.* **257**, 13404–13412
- Scherer, G., Schmid, W., Strange, C. M., Roewekamp, W. & Schütz, G. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7205–7208
- Seglen, P. O. (1976) *Methods Cell Biol.* **13**, 29–83
- Tilghman, S. M., Ballard, F. J. & Hanson, R. W. (1976) in *Gluconeogenesis: Its Regulation in Mammalian Species* (Hanson, R. W. & Mehlman, M. A., eds.), pp. 47–91, John Wiley, New York
- Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412
- Wicks, W. D. (1974) *Adv. Cyclic Nucleotide Res.* **4**, 335–438
- Wicks, W. D., Kenney, F. T. & Lee, K. L. (1969) *J. Biol. Chem.* **244**, 6008–6013
- Yoo-Warren, H., Monahan, J. E., Short, J., Short, H., Bruzel, A., Wynshaw-Boris, A., Meisner, H. M., Samols, D. & Hanson, R. W. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3656–3660