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Regulation of Hepatic 6-Phosphofructo-2-kinase/Fructose 2,6-Bisphosphatase Gene Expression by Glucagon*

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The control of hepatic 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase gene expression by glucagon was studied. Intraperitoneal administration of glucagon rapidly decreased the fructose 2,6-bisphosphate content by phosphorylation of 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase and diminution of its V_{max}. Immunologic studies using a specific liver antibody showed that the amount of enzyme rapidly decreased. Northern blot analysis showed that the isozyme expressed is the adult liver form. The 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase mRNA content decreased, whereas that of phosphoenolpyruvate carboxykinase increased, and that of albumin did not change. Run-on transcription assays with isolated nuclei showed inhibition in the relative transcription rate of the 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase gene and a stimulation of phosphoenolpyruvate carboxykinase gene. The regulation of mRNA stability of 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase by glucagon was also studied. The half-life of mRNA decreased in the presence of glucagon, suggesting that proteins modulated by a glucagon-dependent process are regulating its stability. The time course of mRNA levels correlated with the transcription inhibition of gene and destabilization of mRNA, indicating that glucagon modulates 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase gene expression at both transcriptional and posttranscriptional levels.

Rat liver 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase (PFK-2/FBPase-2; EC 2.7.1.105/3.1.3.46)[1] is encoded by a gene whose expression is subject to a complex pattern of hormonal regulation (1). The bifunctional enzyme PFK-2/FBPase-2 catalyzes both the synthesis and degradation of Fru-2,6-P_2, stimulator of 6-phosphofructo-1-kinase and inhibitor of fructose 1,6-bisphosphatase. Since these enzymes catalyze reciprocal reactions in the fructose 6-phosphate/fructose 1,6-bisphosphate substrate cycle, the level of Fru-2,6-P_2 modulates the carbon flow through the coupled metabolic pathways of glycolysis and gluconeogenesis in mammalian liver. PFK-2/FBPase-2 activities are regulated by substrates and effectors and by phosphorylation/dephosphorylation by cAMP-dependent protein kinase (1-3). Glucagon, acting via cAMP-dependent protein kinase, causes the phosphorylation of hepatic PFK-2/FBPase-2. The phosphorylation results in the activation of FBPase-2 and in the inactivation of PFK-2, producing the disappearance of Fru-2,6-P_2 from the liver cell (1-3). A similar situation has been found during starvation (1-4), diabetes (1-5), and after partial hepatectomy (6), Fru-2,6-P_2 being a good marker of hepatocyte metabolic state. In these situations, the gene expression of PFK-2/FBPase-2 is also modulated. The amount of the bifunctional enzyme decreased (6-8), whereas its mRNA levels were not modified during starvation (7) or decreased in diabetes (8) and after partial hepatectomy (9). The Fru-2,6-P_2 content, the amount of enzyme, and its mRNA were restored or increased by refeeding a high carbohydrate diet (7), by insulin administration (7, 8), or through the regenerative process (6, 9), respectively. It seems obvious that in all these situations glucagon has an important role in the enzyme regulation. However, the mechanisms by which the glucagon affects the expression of PFK-2/FBPase-2 gene have been only partially studied. In rat hepatoma cells (10) and in primary culture of hepatocytes (11), dibutyril cAMP prevented the increase in PFK-2/FBPase-2 mRNA induced by insulin or glucocorticoids.

In order to study the effects of glucagon on the regulation of gene expression of PFK-2/FBPase-2, we have injected glucagon into rats intraperitoneally and analyzed its effects on the activity, amount of enzyme, mRNA levels, transcription rate, and mRNA stability of PFK-2/FBPase-2. We report here that glucagon modulates the PFK-2/FBPase-2 gene expression at both transcriptional and posttranscriptional levels.

EXPERIMENTAL PROCEDURES

Materials—[α-^{32}P]dCTP (3000 Ci/mmol), [α-^{32}P]UTP (3000 Ci/mmol), [5,6-^{3}H]uridine (35-50 Ci/mmol) were from Amersham Corp. The random primed DNA labeling kit and restriction endonucleases were from Boehringer Mannheim. N-hybond membranes were from Amersham. Fetal calf serum and cell culture media were from Life Technologies, Inc. Enzymes and other biochemical reagents were either from Boehringer Mannheim or Sigma. All chemicals were of analytical grade. Rapid glucagon and long acting glucagon (zinc-protamine-glucagon; kindly supplied by Dr. Axel Kahn, Institut de la Santé et de la Recherche Médicale, Paris) were from Novo Ind.

Animals—Fed male Sprague-Dawley rats (180-220 g) were subjected to a 12-h light/12-h dark cycle (light periods starting at 08:00 h). Rats were injected at 8 a.m. intraperitoneally with both rapid glucagon (1 mg/kg) and with long acting glucagon (zinc-protamine-glucagon, 5.4 mg/kg) in order to obtain a rapid and sustained hormonal impregnation (12). Control rats were injected with 0.15 M NaCl solution. The animals were killed by decapitation. Liver was removed and quickly freeze-clamped and placed into liquid nitrogen.

Metabolite and Enzyme Assays—Fru-2,6-P_2 was extracted and measured as described by Van Schaftingen *et al.* (13). PFK-2 activity

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[1] The abbreviations used are: PFK-2, 6-phosphofructo 2-kinase (EC 2.7.1.105); FBPase-2, fructose 2,6-bisphosphatase (EC 3.1.3.46); Fru-2,6-P_2, fructose 2,6-bisphosphate; PEPCK, phosphoenolpyruvate carboxykinase (EC 4.1.1.32); kb, kilobase.

was measured at pH 8.5 (V_{max}) and at pH 6.5, as described by Bartrons *et al.* (14), after partial purification of the extract with polyethylene glycol 6000 (6–21%). The PFK-2 activity ratio (pH 6.5/pH 8.5) is a measure of the phosphorylation state of the bifunctional enzyme (14). The protein concentration was determined according to Bradford (15), using bovine serum albumin as standard.

Western Blot Analysis—Immunoblot analysis was performed essentially as described by Burnette (16) with a 1:200 dilution of polyclonal antibody raised against a synthetic decapeptide (GELTQTRLQK), corresponding to the N terminus of rat liver PFK-2/FBPase-2 (17). This liver-specific antibody was a gift from Louis Hue (Louvain University, Belgium). Bound antibodies were detected by incubation with ^{125}I-labeled Protein A (1–2 × 10^6 cpm/ml) for 30 min, and, after washing, the film was exposed to x-ray film. The amount of enzyme was evaluated by densitometric scanning of the autoradiograms using an LKB Ultroscan XL laser densitometer and GelScan XL (2.1) software.

RNA Isolation and Northern Blot Analysis—Total RNA was extracted from frozen rat tissues by the LiCl/urea method (18). Northern blot analysis was carried out using standard procedures (19). The following probes were used: a 1.4-kb *Eco*RI fragment isolated from the cDNA for PFK-2/FBPase-2, common probe (7); a 0.3-kb *Eco*RI/*Ban*II fragment isolated from the first exon for PFK-2/FBPase-2, liver-specific probe (9); a 2.6-kb *Pst*I fragment from cDNA clone (pPCK10) for PEPCK (20); and a 1.1-kb *Pst*I fragment isolated from cDNA clone (pRSA 13) for albumin (21). All DNA probes were generated by labeling with [α-^{32}P]dCTP to a specific radioactivity of ≈1.5 × 10^9 cpm/μg of DNA by random priming with Klenow DNA polymerase. The mRNA levels were evaluated by densitometric scanning of the autoradiograms.

Isolation of Liver Nuclei and Run-on Transcription Analysis—Nuclei were isolated from liver by a modification of the method of Laitinen *et al.* (22). Fresh livers were homogenized with a Potter-Elvehjem teflon-glass homogenizer in 10 volumes of STM buffer (250 mM sucrose, 50 mM Tris-HCl, pH 7.4, 5 mM $MgSO_4$) containing 0.1 mM phenylmethylsulfonyl fluoride and 0.5 μg/ml aprotinin, filtered though four layers of cheesecloth, and centrifuged at 800 × *g* for 10 min at 4 °C. The pellet was resuspended in the same buffer and sedimented again at 800 × *g* for 5 min at 4 °C. The pellet was resuspended in RSB buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$) containing 0.1 mM phenylmethylsulfonyl fluoride and 0.5 μg/ml aprotinin, and the cells were lysed by stepwise addition of 10% Nonidet P-40 to a final concentration of 0.5% with gentle vortexing (≈30 s). Detergent extraction was repeated twice. Nuclei were then centrifuged at 800 × *g* for 5 min at 4 °C and washed three times with RSB buffer without detergent. The final pellet was resuspended in nuclei storage buffer (40 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 0.1 mM EDTA, and 40% glycerol) and stored at −80 °C. Intact nuclei without cytoplasmic remnants, as revealed by phase-contrast microscopy, were thus obtained. The entire isolation procedure was completed in 40 min.

The run-on transcription reaction in isolated nuclei was carried out at 30 °C for 20 min using the reaction mixture described (23). Twenty million nuclei were used per assay in a total reaction volume of 0.2 ml containing 200 μCi of [α-^{32}P]UTP (specific activity 3000 Ci/mmol). Labeled RNA was extracted from the reaction mixture and resuspended in prehybridization solution essentially as described previously (24). Labeled RNA products were hybridized to nylon membrane containing the liver-specific genomic 1.6-kb *Eco*RI/*Xba*I fragment for PFK-2/FBPase-2 (10). The PEPCK DNA was the pPCK-B7.0 genomic clone (kindly supplied by Dr. Richard W. Hanson, Case Western Reserve University, Ohio) (20). pBS vector was used as control for background hybridization. Prior to hybridization, DNAs (4 μg/lane) hybridized to nylon membrane were incubated for 6 h at 42 °C in the prehybridization solution. The same amount of radioactivity was added to each hybridization. The filters were washed as described (25). Autoradiography and densitometer scanning were carried out as with Northern blots.

Isolation and Incubation of Hepatocytes—Hepatocytes were prepared from male rats (14). Isolated hepatocytes (4 × 10^6 cells/vial in a final volume of 2 ml) were preincubated with shaking for 30 min at 37 °C in Krebs-Henseleit bicarbonate buffer, which was equilibrated with O_2/CO_2 (19:1) at pH 7.4 and contained 10 mM glucose and 1% bovine serum albumin. Actinomycin D (5 μg/ml) was added after the preincubation. Glucagon (10^{-7} M) was added where indicated. At the appropriate times, samples of the cell suspension were removed and centrifuged at 350 × *g* for 5 min at 4 °C. The supernatants were discarded and the pellets frozen in liquid nitrogen. RNA extraction and Northern blot analysis were carried out as described above. The viability of hepatocytes was monitored by trypan-blue exclusion, and was always greater than 90%.

Pulse-Chase Analysis—Isolated hepatocytes were plated with Williams' E medium supplemented with fetal calf serum (5%, v/v), glutamine (1 mM), gentamycin (50 μg/ml), insulin (1 nM), and dexamethasone (1 nM) at a plating density of ~6 million cells/plate (5 ml/plate). After 3 h, the plating medium and unattached cells were removed and the cells incubated with fresh medium and dexamethasone (1 μM) for 16 h. After this time, the cells were exposed for 3 h to [5,6-^{3}H]uridine (0.15 mCi/plate, 1 μM final concentration) to label cellular RNA. To determine radioactive labeling, the cells were rinsed twice with medium and incubated with fresh medium containing 5 mM unlabeled uridine and cytidine and in the absence or presence of glucagon (10^{-7} M), cycloheximide (10 μg/ml), and cycloheximide-glucagon (10 μg/ml and 10^{-7} M), respectively. At the indicated times, the medium was decanted, and the cells were lysed directly with a solution containing 6 M urea and 3 M LiCl (18) and the total RNA extracted as described above. Labeled RNA (10^6 cpm for 100 μg in 1 ml) were hybridized to N-hybond (Amersham) membranes carrying slot spots of linearized PFK-2/FBPase-2 cDNA (4 μg/lane). Linearized pBS plasmid DNA (4 μg/lane) was used as negative control for nonspecific hybridization. Hybridization was carried out for 72 h. The membranes were washed at room temperature with 0.1 × SSC (20 × SSC is 3 M NaCl and 0.3 M trisodium citrate at pH 7.4) and 0.1% SDS for 5 min; 20 min at 50 °C with 0.1 × SSC and 0.1% SDS; 20 min at 37 °C with 0.1 × SSC and RNase A (10 μg/ml); 20 min at 50 °C with 0.1 × SSC and 0.1% SDS. Slots were excised and then counted with 10 ml of F-1 Normascint mixture (Scharlau, Spain) in a liquid scintillation counter (LS 5000CE Beckman Instruments, Scotland).

RESULTS

Effect of Glucagon Administration on Fru-2,6-P_2 Levels and on the Amount and Activity of PFK-2/FBPase-2

Rats were injected intraperitoneally with both rapid and long acting glucagon in order to obtain a rapid and sustained hormonal impregnation (12). In this situation, the Fru-2,6-P_2 levels decreased rapidly (from 8.7 nmol/g to 1.4 nmol/g at 5 min) in parallel to the phosphorylation and inactivation of bifunctional enzyme by cAMP-dependent protein kinase. The PFK-2 activity (V_{max}), and its activity ratio decreased at 5 min and remained low during the studied time (Fig. 1). One could argue that the differences found were due to stress by animal manipulation and/or to differences by circardian cycle. To rule out this possibility, we analyzed the Fru-2,6-P_2 levels, PFK-2 activity, and its activity ratio from animals injected with a NaCl physiological solution. Neither the Fru-2,6-P_2 levels, nor the PFK-2 activity were modified (Fig. 1). To determine whether the amount of bifunctional enzyme changed after glucagon administration, we used immunoblotting with a specific antibody raised against an N-terminal decapeptide specific to liver isozyme (17). We observed a decrease in the protein level at 5–10 min, the content remaining low after this time. Since the specific antibody equally recognized the nonphosphorylated and the phosphorylated forms of the enzyme (see Ref. 9 and results not shown), the decrease in immunoreaction cannot be due to the phosphorylation of the enzyme by cAMP-dependent protein kinase (Fig. 2).

Effect of Glucagon on the Expression of PFK-2/FBPase-2 mRNA

The relative abundance of PFK-2/FBPase-2 mRNA was determined by Northern blot hybridization of total RNA extracted from livers at various times following glucagon administration. We used a 1.4-kb cDNA probe (7) that recognized the mRNAs of the different isozymes of PFK-2/FBPase-2 (25). A decrease in PFK-2/FBPase-2 mRNA was observed after the first minutes, with a minimum at 120 min,

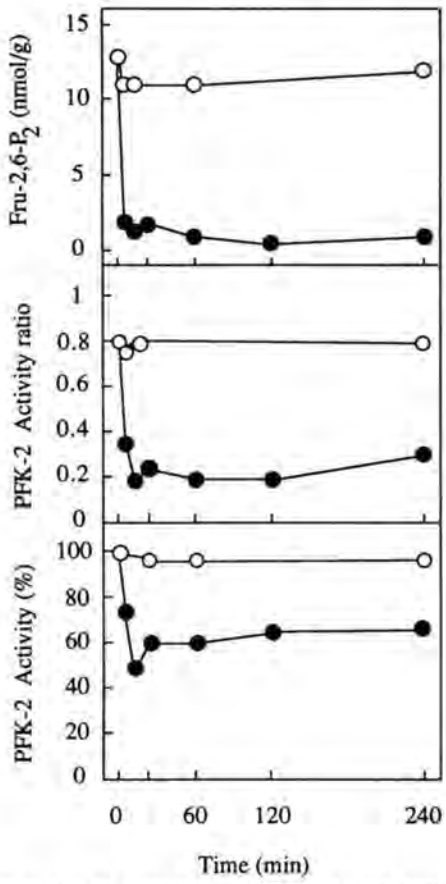

FIG. 1. **Fru-2,6-P_2 content and PFK-2 activity.** Fru-2,6-P_2 content, PFK-2 activity ratio, and PFK-2 activity from glucagon treated animals (●) or 0.15 M NaCl saline solution (○) are shown. Each point shows the mean for three to five rats.

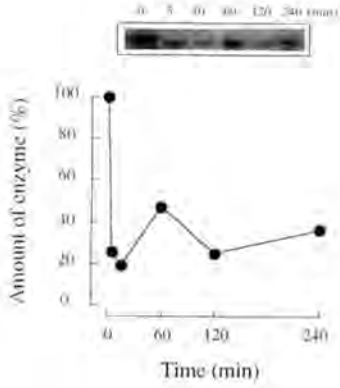

FIG. 2. **Amount of PFK-2/FBPase-2 after glucagon administration.** Enzyme protein was measured after fractionation with polyethylene glycol 6000 (6–21%). Thirty μg of protein was used/lane for 10% SDS-polyacrylamide gel electrophoresis and then transferred to nylon membranes and incubated with anti-PFK-2/FBPase-2 liver-specific antibody. The intensity of the autoradiographic signal was quantified by laser densitometry and presented as a percentage of the signal obtained at time zero, which was considered as 100%. A representative experiment is shown. The experiment was repeated three times with similar results.

which was maintained after 240 min (Fig. 3). As control of this experiment, we analyzed the content of PEPCK and albumin mRNAs, since an increase of PEPCK mRNA content had previously been reported in the presence of high levels of

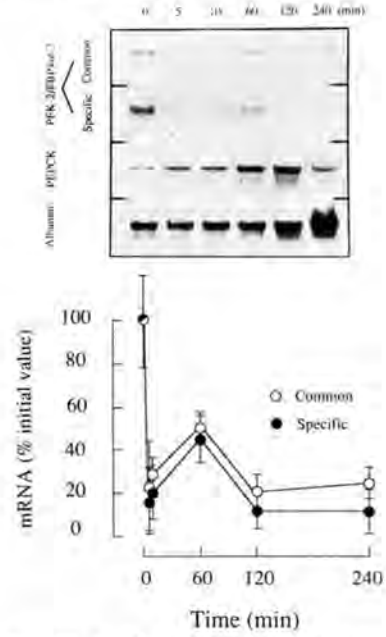

FIG. 3. **Time course of mRNA accumulation in glucagon-treated animals.** Total RNA (20 μg/lane) extracted from normal (0 h) and glucagon-treated rat livers at different times were transferred to nylon membranes after electrophoresis in 1% agarose. The integrity of the RNA and the equivalence of inputs were verified by observing the rRNA bands in the ethidium bromide-stained gels under UV irradiation. The blots were hybridized serially to four different probes as follows: PFK-2/FBPase-2 (common (○) and liver-specific (●)), PEPCK, and albumin cDNAs, as described under "Experimental Procedures." After each hybridization, the probe was eluated by washing the blot in 0.1 × SSC at 90–100 °C for 30 min. The intensity of the autoradiographic signal was quantified by laser densitometry and presented as a percentage of the signal obtained at time zero, which was considered as 100%. Data are means ± S.E. from three to four different animals. Representative Northern blots are shown.

glucagon or cAMP (1, 26–29), whereas the albumin mRNA levels were unmodified (9, 30). As control of the stress by animal manipulation and/or differences by circardian cycle, we analyzed PFK-2/FBPase-2 mRNA content from animals injected with 0.15 M NaCl solution, not observing variations in the mRNA content during the times analyzed (results not shown).

Since the 1.4-kb probe hybridizes with mRNAs of different isozymes (25), we measured the content of liver-specific mRNA by using a specific 0.3-kb cDNA probe (9). This probe contains the entire coding region of the first exon of liver-specific transcript, including the nucleotides that encode the decapeptide corresponding to the N terminus of liver PFK-2/FBPase-2. The pattern of expression was similar to that found with the 1.4-kb probe (Fig. 3), suggesting that the isozyme expressed is the adult liver form.

Transcriptional Activity of the PFK-2/FBPase-2 Gene after Glucagon Administration

To determine whether the change in the levels of PFK-2/FBPase-2 mRNA after glucagon administration was due to a variation in the transcription rate of the PFK-2/FBPase-2 gene, we performed a series of nuclear run-on assays. Table I shows that the transcription rate of the rat liver PFK-2/FBPase-2 gene decreased after glucagon administration in parallel to the decrease of mRNA (Fig. 3). The inhibitory effect of glucagon on PFK-2/FBPase-2 gene transcription was rapid (≈71% of inhibition at 10 min), recovering at 240 min. As control of this experiment, we measured the transcription

TABLE I

Transcriptional activity of the PFK-2/FBPase-2 and PEPCK genes after glucagon administration

Nuclear run-on assays were performed on equal numbers of nuclei (2×10^7) isolated from the rat liver at the indicated times after glucagon administration. Since the incorporation of counts varied with each nuclear preparation, hybridization was carried out with equal numbers of counts per each set. *In vitro* extended ^{32}P-labeled RNA transcripts were hybridized to 4-μg samples of the indicated cDNAs immobilized on nylon membranes. Hybridization was carried out as it is described under "Experimental Procedures." Autoradiographs were quantified by laser densitometry and are expressed as arbitrary units after subtraction of the values obtained for vector DNA. Percent values were calculated by assigning a value of 100 to the rate at $t = 0$. Data are means ± S.E. from three rats.

Time	Relative rate of gene transcription			
	PFK-2/FBPase-2		PEPCK	
min	*units*	*%*	*units*	*%*
0	1.7 ± 0.1	100	3.1 ± 0.6	100
5	0.8 ± 0.2	47	6.9 ± 0.9	225
10	0.5 ± 0.1	29	5.4 ± 0.1	174
15	0.2 ± 0.1	12	9.4 ± 0.2	303
120	0.8 ± 0.2	47	6.3 ± 0.4	203
240	1.5 ± 0.1	88	4.5 ± 0.3	145

rate of PEPCK gene, since its induction by cAMP had been reported (27–29, 31). As can be seen on Table I, an increase of the transcription rate was observed after glucagon administration, and this remained high during the period studied.

Regulation of PFK-2/FBPase-2 mRNA Half-life by Glucagon

Effect of Cycloheximide—The glucagon regulation of PFK-2/FBPase-2 mRNA content at the transcriptional level does not preclude regulation at other pretranslational levels. For example, cAMP regulates both the rate of transcription of PEPCK and the stability of its mRNA (27–29, 31, 32). The possible role of glucagon in controlling the degradation rate of PFK-2/FBPase-2 mRNA was estimated from the decay rate of the mRNA in hepatocytes incubated in media containing actinomycin D (5 μg/ml) and in the presence or absence of glucagon (10^{-7} M). As seen in Fig. 4*A*, the half-life of PFK-2/FBPase-2 mRNA was ≈2.5 h, agreeing with data previously reported (9–11). The presence of glucagon increased the half-life of mRNA (Fig. 4*A*). As a control, we analyzed the expression of PEPCK, known to be stabilized by glucagon (32). As shown in Fig. 4B, the PEPCK mRNA levels were stabilized in the presence of glucagon. The rapid decrease of the PFK-2/FBPase-2 mRNA content after glucagon administration *in vivo* (Fig. 3) does not correlate with the half-life of PFK-2/FBPase-2 mRNA obtained with rat hepatocytes in the presence of glucagon (Fig. 4*A*), suggesting that the half-life obtained in these conditions does not reflect the half-life of PFK-2/FBPase-2 mRNA *in vivo*. One could argue, as described by other mRNAs (33–35), that actinomycin D could be affecting PFK-2/FBPase-2 mRNA half-life. To answer this question we have used pulse-chase protocols to directly assess PFK-2/FBPase-2 mRNA half-life in hepatocytes in the presence or absence of glucagon (10^{-7} M). As shown in Fig. 5, glucagon decreased the half-life of PFK-2/FBPase-2 mRNA from ≈2.5 h to ≈15 min, correlating this decrease with the low content of mRNA found after glucagon administration (Fig. 3).

The stability of certain mRNAs is known to be affected by translation (35). Cycloheximide, an inhibitor of translation, causes stabilization of some mRNAs. This effect has been attributed to rapid loss of an unstable protein involved in the cleavage of these mRNAs (36). With this possibility in mind, we analyzed the half-life of PFK-2/FBPase-2 mRNA in the

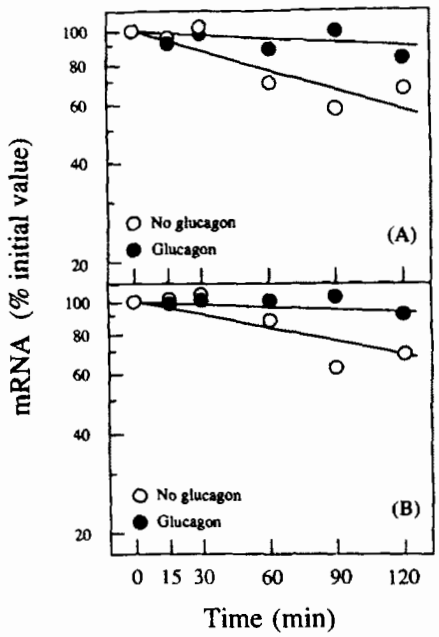

FIG. 4. **Effects of glucagon in the decay of PFK-2/FBPase-2 and PEPCK mRNAs in isolated hepatocytes.** Hepatocytes were isolated from rat liver and incubated at 37 °C as described under "Experimental Procedures." All cells were incubated in the presence of actinomycin D (5 μg/ml) for the times indicated. ●, hepatocytes were incubated with glucagon (10^{-7} M). At the times indicated, the total RNA was extracted and transferred to nylon membranes after electrophoresis in 1% agarose and hybridized with PFK-2/FBPase-2 (common probe) (*A*) and PEPCK (*B*) cDNAs, as described under "Experimental Procedures." The mRNA levels were quantified by laser densitometry of autoradiograms and expressed relative to the amount present at time zero, which was taken as 100%. Data are means from three to four different animals.

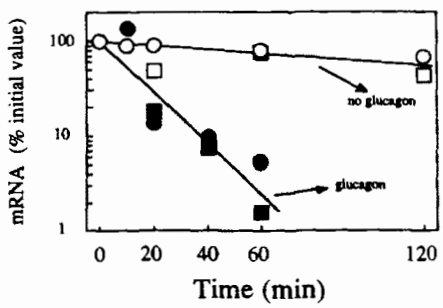

FIG. 5. **Effects of glucagon, cycloheximide, and cycloheximide-glucagon on PFK-2/FBPase-2 mRNA stability as measured by pulse-chase.** Hepatocytes were incubated for 3 h with [5,6-^{3}H]uridine (30 μCi/ml). After extensive rinsing, cells were incubated with fresh medium containing 5 mM unlabeled uridine and cytidine in the absence (○, ●) or presence (□, ■) of 10 μg/ml cycloheximide and in the absence (○, □) or presence (●, ■) of 10^{-7} M glucagon for the indicated times. Labeled total RNA was then isolated. Incorporation of labeled nucleotides at $t = 0$ was 11,700 cpm/μg RNA, maintaining constant during the time studied and in the different conditions. Labeled RNA was hybridized to N-hybond membranes carrying slot spots of linearized PFK-2/FBPase-2 and pBS plasmid DNAs. The selection of mRNA is indicated under "Experimental Procedures." The amount of radioactivity hybridizing to PFK-2/FBPase-2 at $t = 0$ was 1.6 cpm/μg RNA, and the nonspecific hybridization with pBS plasmid was 0.3 cpm/μg RNA, the difference being (1.3 cpm/μg RNA) the specific for PFK-2/FBPase-2, which was considered as 100%.

presence of cycloheximide (10 μg/ml) and cycloheximide-glucagon (10 μg/ml and 10^{-7} M) in hepatocytes using pulse-chase protocols. As shown in Fig. 5, cycloheximide in the

presence or absence of glucagon did not modify the half-life of PFK-2/FBPase-2 mRNA.

DISCUSSION

Glucagon induces a series of metabolic changes in the liver that converts the hepatocyte into a net producer of glucose. Glucagon exerts its action via the formation of cAMP and the activation of cAMP-dependent protein kinase. PFK-2/FBPase-2 is phosphorylated by cAMP-dependent protein kinase, and this phosphorylation results in the activation of phosphatase and in the inactivation of kinase activities, causing the disappearance of Fru-2,6-P_2 from the liver cell (1–3). The results reported herein show that the decrease in Fru-2,6-P_2 concentration is also due, in part, to a decrease in the amount of bifunctional enzyme.

Glucagon rapidly down-regulated PFK-2/FBPase-2 gene transcription and steady-state mRNA levels (Table I and Fig. 3). This pattern of hormonal regulation is similar to that of hepatic glycolysis regulatory enzymes and opposite to that of gluconeogenesis (37). Thus, while glucokinase and pyruvate kinase mRNA levels decreased in rat liver after glucagon or cAMP administration, as a consequence of the decrease in their transcription rates (12, 38), the PEPCK mRNA content increased due to the increase in its transcription rate (26–29, 31) (Table I and Fig. 3). This modulation indicates the coordinate hormonal regulation of gene expression of hepatic glycolytic/gluconeogenic enzymes (37, 39). cAMP prevented the increase in PFK-2/FBPase-2 mRNA induced by insulin or glucocorticoids in rat hepatoma cells (10) and in primary culture of hepatocytes (11). The decrease in PFK-2/FBPase-2 mRNA levels in parallel to the inhibition of the transcription rate of the PFK-2/FBPase gene seems to indicate that glucagon regulates the PFK-2/FBPase-2 mRNA content *in vivo* at the transcriptional level. This inhibition of the transcription rate could explain the decrease observed in the PFK-2/FBPase-2 mRNA levels in situations with high glucagon/insulin ratio, like after partial hepatectomy (9) or during diabetes (8).

The ability of glucagon to decrease PFK-2/FBPase-2 gene transcription is probably mediated by a hormone response element. Recently, Lange *et al.* (40) have identified a glucocorticoid response element in the first intron of the gene of skeletal muscle/liver PFK-2/FBPase-2, which may explain the regulation of gene expression of PFK-2/FBPase-2 by glucocorticoids (41, 42). No consensus sequence known for cAMP response elements has been identified in the PFK-2/FBPase-2 gene. A similar situation could be applied to insulin. Although it is known that the insulin increases PFK-2/FBPase-2 gene transcription, probably also via a hormone response element, no consensus sequence has been found (40). Further studies are necessary to identify these putative glucagon and insulin response elements.

Little is known about the mechanism of mRNA degradation. Evidence suggests that the 3′-polyadenosine (poly(A)) tail may be involved in the regulation of the mRNA turnover. The elongation of poly(A) tails of certain mRNAs correlates with their stabilization (35). Poly(A) tail-binding proteins interact with the poly(A) tail preventing deadenylation and subsequent degradation of mRNAs *in vitro* (43). A repeated AUUUA sequence in the 3′-nontranslated region appears to be responsible for the destabilization of some mRNAs (44–46). RNA folding also seems to have a role in mRNA stability, since it has been shown that a stem loop in the 3′ terminus of the mRNA stabilized mRNAs of the transferrin receptor (47, 48) and histone (49). Modification of the length of hepatic PFK-2/FBPase-2 mRNA has not been observed (7, 9, 10, 41, 42) nor has an AUUUA sequence been found in its 3′-nontranslated region (7, 50), and no study of RNA folding has been reported. No alteration in the degradation rate of PFK-2/FBPase-2 mRNA was observed when rat hepatoma cells were treated with insulin (10). Kummel and Pilkis (11) described that cAMP decreased the stimulatory effect of dexamethasone at early times and increased the PFK-2/FBPase-2 mRNA content after long incubation periods in culture of rat hepatocytes, suggesting to these authors that cAMP could affect PFK-2/FBPase-2 mRNA stability. It is apparent from the data presented here that glucagon down-regulated PFK-2/FBPase-2 mRNA (Fig. 3) not only at the level of gene transcription (Table I) but also decreasing its half-life (Fig. 5). A similar mRNA degradation rate in the presence of glucagon has been previously reported for glucokinase (38). In contrast to the rapid disappearance of PFK-2/FBPase-2 mRNA in the pulse-chase experiments, its apparent rate of decay after addition of the transcriptional inhibitor actinomycin D was much slower, raising the possibility that a short lived RNA and/or protein may be involved in the rapid turnover process. A similar suggestion comes from other studies (38).

In conclusion, the results reported herein show a regulation of PFK-2/FBPase-2 gene expression by glucagon at both the transcriptional and posttranscriptional levels.

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