

Role of cysteine in the dietary control of the expression of 3-phosphoglycerate dehydrogenase in rat liver

Younes ACHOURI, Mariette ROBBi and Emile VAN SCHAFTINGEN¹

Laboratory of Physiological Chemistry, ICP and Université Catholique de Louvain, BCHM 7539, Avenue Hippocrate 75, B-1200 Brussels, Belgium

Shifting rats to a protein-free, carbohydrate-rich diet, although not starvation, resulted in the appearance of mRNA for, and activity of, 3-phosphoglycerate dehydrogenase (3-PGDH) in liver as well as in a marked decrease in plasma cystine concentration. Refeeding with protein caused a 50% decrease in the mRNA in 8 h and its complete disappearance within 24 h, followed by a slower disappearance of the enzymic activity. Intraperitoneal administration of cysteine or methionine to protein-starved rats decreased the mRNA by 50–60% after 8 h. However, the repeated administration of cysteine failed to cause the complete disappearance of this mRNA in 24 h. In hepatocytes in primary culture, cysteine plus methionine and glucagon had, independently, an approx. 4-fold inhibitory effect on the abundance of the 3-PGDH mRNA and caused its almost complete disappearance when tested together. Insulin had an approx. 2-

fold stimulatory effect, which was antagonized by cysteine plus methionine but was still apparent in the presence of glucagon. Nuclear run-on experiments and analysis of the stability of the mRNA with 5,6-dichlorobenzimidazole riboside, an inhibitor of RNA polymerase II, suggested that the effect of cysteine plus methionine was due to destabilization of the mRNA, whereas the effect of glucagon was exerted on transcription. Cysteine, but not methionine, inhibited the accumulation of 3-PGDH mRNA in FTO2B hepatoma cells. In conclusion, the dietary control of the expression of the 3-PGDH gene in liver seems to involve the negative effects of cysteine and glucagon and the positive effect of insulin.

Key words: glucagon, plasma amino acid, protein starvation, serine biosynthesis.

INTRODUCTION

The first step of serine synthesis is catalysed by 3-phosphoglycerate dehydrogenase (3-PGDH), an enzyme that is widely distributed in organisms and in tissues. In rat liver, the activity of this enzyme is largely dependent on nutritional status. It is nearly inactive in animals fed with a normal, protein-containing diet but increases more than 10-fold after they are shifted to a protein-free, carbohydrate-rich diet [1,2]. Studies with diets containing different mixtures of amino acids have shown that four essential amino acids (methionine, tryptophan, threonine and possibly valine) and cystine participate in the repression of this enzyme [2].

We have previously cloned the cDNA encoding rat liver 3-PGDH and shown that the corresponding mRNA is widely distributed in tissues and that its abundance in the liver, although not in other tissues, is highly dependent on nutritional status [3]. This mRNA is indeed undetectable in the livers of rats fed with a normal, protein-containing diet but is fairly abundant when the rats have been maintained for 3 days on a high-carbohydrate, protein-free diet (corn starch) [3].

The purpose of the present work was to understand the mechanism of this dietary effect on the expression of 3-PGDH and most particularly to assess the role of several amino acids as well as those of glucagon and insulin on the expression of this gene. Owing to the complexity of humoral and hormonal changes that can be induced by the administration of nutrients or hormones to animals, part of the study was performed on cells in culture, either hepatocytes in primary culture or hepatoma cells.

EXPERIMENTAL

Animals and diets

For the dietary studies, 3-month-old male Wistar strain rats weighing approx. 270–300 g were used. They were fed *ad libitum* on laboratory chow (A03, containing 22% protein, 4.3% lipids and 52% carbohydrates; from UAR, Epinay-sur-Orge, France) or on corn starch (protein-free, carbohydrate-rich diet), or starved as indicated, and kept in a 12 h light/12 h dark regime. When indicated, amino acids were injected intraperitoneally as 0.5 M solutions except for tryptophan and methionine, which were injected respectively as 0.125 and 0.25 M solutions. Rats were killed by decapitation. Livers were removed immediately and frozen in aluminium clamps precooled in liquid N₂ [4]. For the determination of the plasma amino acid concentration, rats were anaesthetized by intraperitoneal injection of 80 mg/kg nembutal; blood was withdrawn from the posterior vena cava and collected in chilled heparinized test tubes. After centrifugation at 1000 g at 4 °C for 10 min, the plasma samples (300 µl) were deproteinized by the addition of an equal volume of a solution containing 0.4 M sulphosalicylic acid and 25 µl of 5 mM D,L-2,4-diamino-n-butyric acid and left to stand at 4 °C for 30 min, then centrifuged at 10000 g for 7 min. The clear supernatants were kept at –20 °C until analysis.

Cell cultures

Hepatocytes were isolated from the livers of fed male Wistar rats by the method of Seglen [5], as modified by Bartrons et al. [6]. The cells were suspended in Dulbecco's modified Eagle's medium

Abbreviations used: DMEM, Dulbecco's modified Eagle's medium; DRB, 5,6-dichlorobenzimidazole riboside; 3-PGDH, 3-phosphoglycerate dehydrogenase.

¹ To whom correspondence should be addressed (e-mail vanschaftingen@bchm.ucl.ac.be).

(DMEM) lacking cystine and methionine and containing 10% (v/v) fetal bovine serum and 0.1 μ M dexamethasone. They were inoculated on Petri dishes (8.5 cm in diameter) at a cell density of approx. 3×10^6 cells per plate and incubated at 37 °C in a humidified atmosphere consisting of air/CO₂ (19:1). After 8 h the medium was replaced by a serum-free medium containing hormones, cysteine and methionine as indicated.

Rat hepatoma FTO2B cells were grown in 75 cm² culture flasks in DMEM/Ham's F12 medium supplemented with 10% (v/v) fetal calf serum and used in the logarithmic phase of growth (5×10^6 cells per flask). They were then incubated under the indicated conditions. To stop the experiments, cells were washed once with PBS (150 mM NaCl/2.5 mM KCl/8 mM Na₂HPO₄/1.5 mM KH₂PO₄) and RNA was extracted and purified by the guanidinium isothiocyanate/CsCl procedure [7].

Enzyme and amino acid assays

3-PGDH was assayed spectrophotometrically at 30 °C in a mixture comprising 25 mM Hepes, pH 7.1, 90 μ M phosphohydroxypruvate, 90 μ M NADH and 400 mM KCl [3]. One unit is defined as the amount of enzyme catalysing the reduction of 1 μ mol of phosphohydroxypruvate in 1 min under these assay conditions. Amino acid concentrations in the plasma were determined with a Biochrom 20 (Pharmacia) amino acid analyser.

RNA extraction and Northern blotting

Total RNA was isolated from rat liver by a guanidinium thiocyanate/CsCl procedure [8], as described [9]. The integrity of the RNA preparations was analysed systematically by electrophoresis and detection of the 18 S and 28 S ribosomal RNA species by ethidium bromide staining; the ratio of 28 S to 18 S RNA was always close to 2, without any detectable low-molecular-mass form. Northern blots were performed on 25 μ g RNA samples, as described [3]. A 25 μ g RNA sample derived from two rats maintained for 8 days on a protein-free diet was loaded on all gels to serve as an internal standard. The probe used, a *Xho*I restriction fragment of approx. 1000 bp, corresponding to the 3' end of the rat 3-PGDH cDNA, was labelled with [α -³²P]dCTP by random priming [7]. The membranes were dried and exposed to a PhosphorImager screen (Molecular Dynamics, Sunnyvale, CA, U.S.A.) for the detection and quantification of the radioactive signals. In all cases the 3-PGDH mRNA appeared as a sharp band corresponding to an approx. 2.1 kb species [3]. The results were normalized with the standard RNA as a reference, to which a value of 1 arbitrary unit was assigned.

Transcription run-on assays

For the transcription run-on assays, the isolation of nuclei, the preparation of the cell lysate and the reaction conditions were the same as described [10]. The isolation of radiolabelled transcripts was performed by the method of Vaultont et al. [11]. Hybridization of the purified transcripts to filter-bound cDNA plasmids was performed as described [10]. Hepatocytes from five 75 cm² culture flasks were pooled for each experimental condition.

RESULTS

Effect of diet on the activity of liver 3-PGDH and on the concentration of its mRNA

Figure 1 shows the time course of the effect of protein starvation on both the activity of 3-PGDH and the concentration of its mRNA, as determined by Northern blot analysis. The 2.1 kb

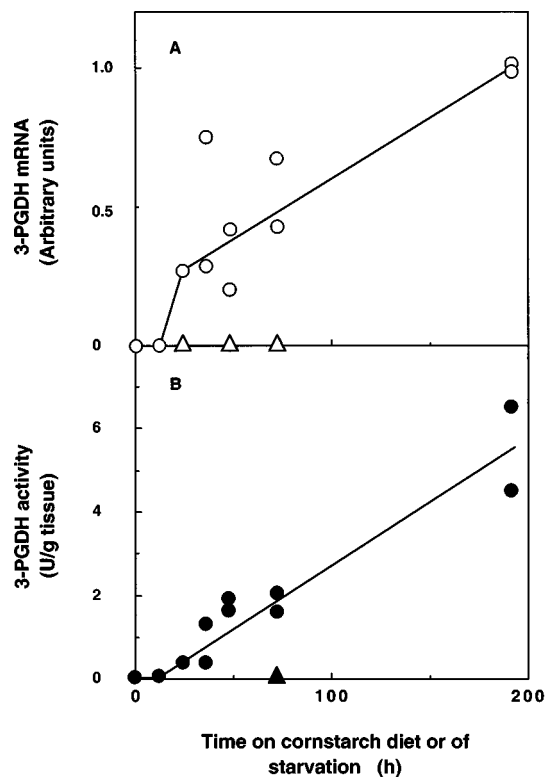


Figure 1 Effect of a corn starch diet or of starvation on the mRNA (A) and the activity (B) of 3-PGDH in rat livers

Each point represents one animal for rats fed with corn starch (○, ●), and three animals for starved rats (△, ▲). U, units.

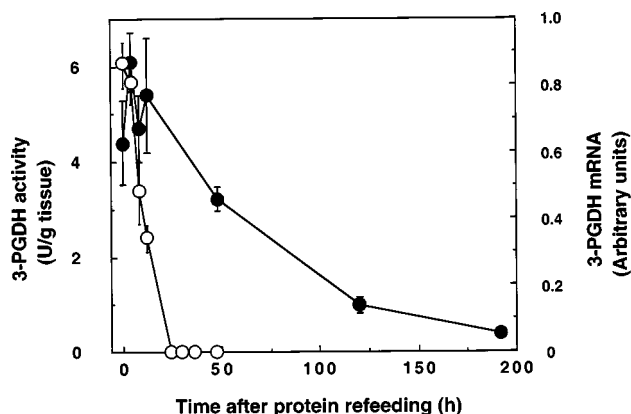


Figure 2 Time-course of the effect of protein administration on 3-PGDH mRNA level (○) and activity (●) in the livers of rats fed with a protein-free, carbohydrate-rich diet for 8 days

At time 0, the rats were fed with laboratory chow. Results shown are means \pm S.E.M. for $n = 3$ animals. U, units.

mRNA encoding this enzyme was undetectable in the livers of rats fed with laboratory chow as well as 12 h after the shift to the protein-free diet; it rapidly increased during the following 12 h to reach an intermediate level between days 1 and 3, and increased further more slowly during the next 5 days. There was at least a 100-fold difference in the amount of mRNA between rats fed

Table 1 Effect of intraperitoneal injection of amino acids and glucagon on the 3-PGDH mRNA level in the livers of rats fed with a protein-free, carbohydrate-rich diet for 8 days

The amino acids and glucagon were injected at the indicated doses and times. The amino acid mixture had the following composition (in mmol): cysteine, 0.25; methionine, 0.19; tryptophan, 0.06; threonine, 0.19; valine, 0.13. Results are means \pm S.E.M. for *n* observations.

Injected compound	Dose (mmol)	Time of administration (h)	Time of killing (h)	3-PGDH mRNA (arbitrary units)	<i>n</i>
Control	—	—	—	0.72 \pm 0.04	19
Valine	0.5	0	8	0.78 \pm 0.18	4
Serine	0.5	0	8	0.66 \pm 0.04	3
Alanine	0.5	0	8	0.60 \pm 0.09	3
Threonine	0.5	0	8	0.52 \pm 0.06	3
Tryptophan	0.5	0	8	0.52 \pm 0.01	3
Methionine	0.5	0	8	0.35 \pm 0.02	4
Cysteine	0.5	0	8	0.28 \pm 0.03	7
Cysteine	0.5 (\times 2)	0, 8	16	0.16 \pm 0.06	4
Cysteine	0.5 (\times 3)	0, 8, 16	24	0.24 \pm 0.04	4
Amino acid mixture	0.82 (\times 5)	0, 4, 7, 11, 21	24	0.02 \pm 0.01	3
Glucagon	8.6 \times 10 ⁻⁵ (\times 2)	0, 4	8	0.36 \pm 0.04	3
Glucagon	8.6 \times 10 ⁻⁵ (\times 2)	0, 4			
Cysteine	0.5	0	8	0.22 \pm 0.02	3

with the control diet and those that had received the corn-starch diet for 8 days. The enzyme activity was also very low in the liver of rats fed with laboratory chow as well as 12 h after the shift to the protein-free diet and then increased progressively during the next 7 days. In marked contrast, complete starvation did not result in the appearance of 3-PGDH mRNA or activity after 3 days, thereby indicating a requirement of carbohydrate for 3-PGDH induction.

Rats that had been fed with the corn-starch diet for 8 days were fed again with laboratory chow for various periods from 0 to 8 days (Figure 2). The amount of 3-PGDH mRNA was decreased to 50% at 8 h after the shift to the protein-containing diet and disappeared completely after 24 h. In contrast, 3-PGDH activity was relatively constant for 1 day, as long as the 3-PGDH mRNA was present, and then decreased slowly with a half-life of approx. 2.5 days.

Effect of the administration of amino acid and glucagon on the 3-PGDH mRNA level

In the experiment illustrated in Table 1, we tested the effect of the five amino acids identified by Mauron et al. [2] as having a role in the expression of 3-PGDH, as well as that of alanine and serine. For this purpose, rats that had been maintained for 8 days on the protein-free diet received 0.5 mmol of amino acids by intraperitoneal injection; the 3-PGDH mRNA was measured 8 h later. As shown in Table 1, cysteine and methionine caused a decrease in the concentration of the 3-PGDH mRNA to approx. 40% and 50% of the initial level. Threonine and tryptophan were less potent, both causing an approx. 25% decrease, whereas serine, alanine and valine were without effect.

When two or three doses of 0.5 mmol cysteine were administered at 8 h intervals, the amount of 3-PGDH mRNA decreased further to approx. 25% of the original level at 16 h but did not disappear completely after 24 h (Table 1). Repeated injection of a mixture of the five amino acids identified by Mauron et al. [2] caused, in 24 h, a decrease in the 3-PGDH mRNA that was almost as complete as that induced by protein refeeding.

The administration of two doses of glucagon 4 h apart to protein-starved rats caused, after 8 h, a decrease in the 3-PGDH

mRNA to 50% of the control value. This effect was partly additive with that of cysteine (Table 1).

Effect of diet on the plasma amino acid concentration

Plasma amino acid concentration was measured at intervals in experiments similar to those shown in Figure 1 and 2. The method used allowed the assay of 18 of the 20 'standard' amino acids, with the exception of tryptophan and asparagine. The amino acids showing the largest relative variations are shown in Figure 3. They can be divided into three groups: (1) cystine, the only one to show a marked decrease (the level of this amino acid fell to 32% of the control value after 3 days and to 23% after 8 days; it was restored to normal 24 h after re-administration of the standard diet); (2) the essential amino acids methionine, valine, threonine (Figure 3), leucine and isoleucine (results not shown), whose concentrations decreased more modestly; (3) the non-essential amino acids serine and alanine, whose concentrations increased 2.2 and 1.6-fold respectively after 8 days on corn starch. Prolonged fasting affected the amino acid levels quite differently: the levels of branched-chain amino acids and of phenylalanine were increased the most (1.5–1.8-fold after 8 days), whereas the concentration of several amino acids were decreased to 60% (proline) or to 70–80% (alanine, cystine, histidine and aspartate) of the fed value after 8 days (Figure 3, and results not shown).

At 4 h after the administration of 0.5 mmol of cysteine to protein-starved rats, the concentration of cystine in the plasma was 75% of the control value, whereas the administration of 0.5 mmol of methionine resulted in an increase in the cystine concentration to 160% of the control value and a significant decrease in the serine levels (Figure 3).

Effect of hormones on the 3-PGDH mRNA levels in hepatocytes

The fact that the administration of carbohydrate (corn starch) was required for the appearance of the 3-PGDH mRNA indicated that hormones involved in blood glucose homeostasis could

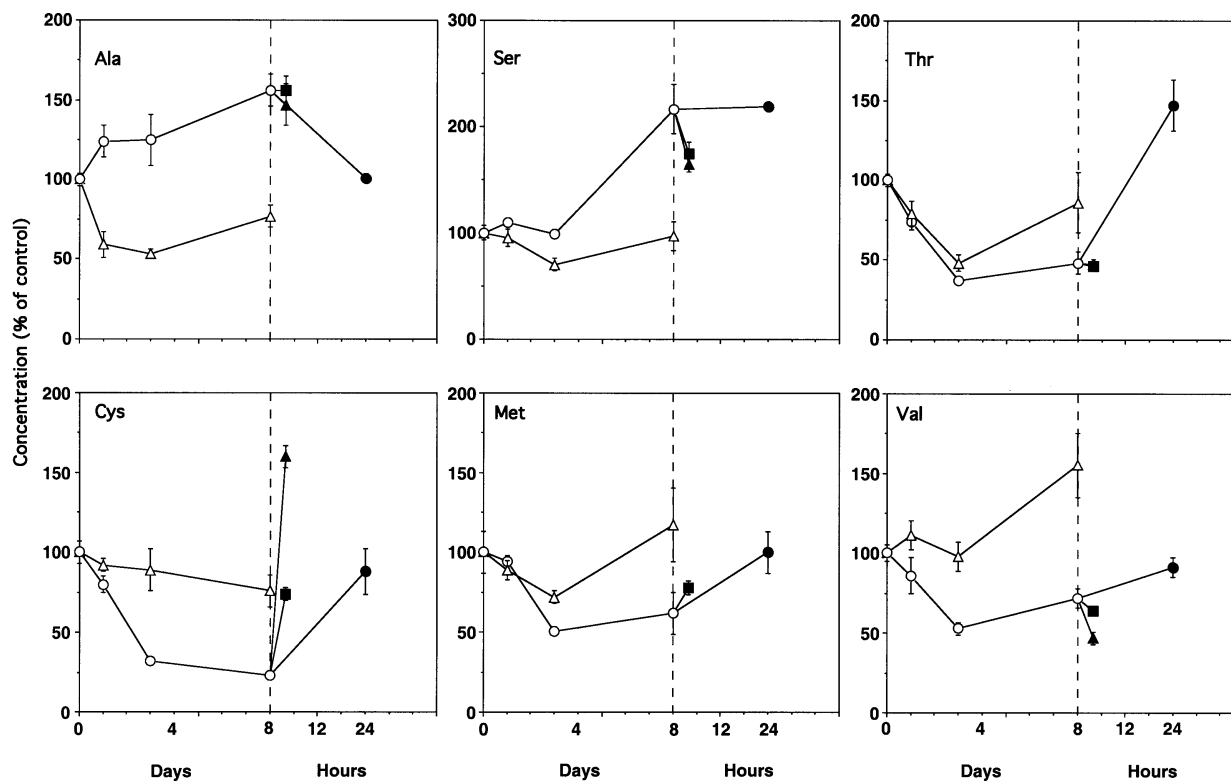


Figure 3 Effect of the protein-free diet, of starvation and of amino acid administration on the concentration of cystine, serine, alanine, threonine, methionine and valine in plasma

Rats were fed with corn starch (○) or starved (△) for the indicated durations. After 8 days on the corn-starch diet, some rats were given laboratory chow (●) or were injected intraperitoneally with 0.5 mmol of cysteine (■) or methionine (▲) and blood samples were taken at the indicated times. Results are expressed as percentages of the concentrations in control rats. These were (in μM): Ala, 752 ± 28 ; Arg, 145 ± 11 ; Asp, 14 ± 2 ; Cys (half-cystine), 92 ± 6 ; Gln, 482 ± 21 ; Glu, 102 ± 4 ; Gly, 423 ± 41 ; His, 53 ± 3 ; Ile, 72 ± 2 ; Leu, 120 ± 5 ; Lys, 435 ± 67 ; Met, 47 ± 6 ; Phe, 55 ± 4 ; Pro, 251 ± 5 ; Ser, 203 ± 14 ; Thr, 219 ± 9 ; Tyr, 100 ± 5 ; Val, 159 ± 8 .

Table 2 Effects of insulin and glucagon on the 3-PGDH mRNA level in hepatocytes cultured in the absence or in the presence of cysteine and methionine

The cells, isolated from a rat fed with laboratory chow, were precultured for 8 h in DMEM lacking cysteine and methionine and supplemented with 10% (v/v) fetal calf serum and 0.1 μM dexamethasone. Then they were washed once with PBS; fresh DMEM, containing 0.1 μM dexamethasone as well as insulin, glucagon, methionine and cysteine at the indicated concentrations, was added. Total cellular RNA was extracted 16 h later and analysed by Northern blotting. Results are means \pm S.E.M. for $n = 3$ values. Similar results were obtained in another experiment. *Significantly different ($P < 0.05$) from the appropriate control without insulin; †significantly different ($P < 0.05$) from the appropriate control without glucagon; ‡significantly different ($P < 0.05$) from the appropriate control without cysteine and methionine.

Condition	3-PGDH mRNA (arbitrary units)	
	Cysteine (140 μM) + Methionine (115 μM) ... -	+
Control	0.460 \pm 0.016	0.100 \pm 0.020‡
Insulin (0.1 μM)	0.810 \pm 0.120*	0.130 \pm 0.003‡
Glucagon (1 μM)	0.080 \pm 0.022†	0.010 \pm 0.010†‡
Insulin plus glucagon	0.230 \pm 0.030*†	0.003 \pm 0.003†‡

participate in control of the expression of the 3-PGDH gene. We therefore tested the effect of insulin and glucagon as well as that of cysteine and methionine on the amount of 3-PGDH mRNA

in hepatocytes derived from rats fed with laboratory chow. As shown in Table 2, a significant amount of 3-PGDH mRNA, comparable to that observed in rat liver after 24 h of corn-starch diet, was present in hepatocytes cultured for 24 h in the presence of DMEM devoid of cysteine and methionine. Cysteine and methionine, as well as glucagon, inhibited the appearance of the 3-PGDH mRNA by at least 75%; their combined effect led to an almost complete blocking of the formation of this mRNA. Insulin had a stimulatory effect that was more marked (1.8-fold) in the absence of sulphur amino acids than in their presence (1.3-fold) and partly antagonized the effect of glucagon in the absence of cysteine and methionine but not in their presence.

Effect of cysteine plus methionine and glucagon on mRNA stability

To investigate the nature of the effects of glucagon and cysteine plus methionine, we determined their effect on the stability of the 3-PGDH mRNA. Hepatocytes were preincubated in serum-free DMEM medium without cysteine and methionine and supplemented with insulin for 16 h to achieve an elevated level of 3-PGDH mRNA. The hepatocytes were then incubated in the presence of 5,6-dichlorobenzimidazole riboside (DRB), an inhibitor of RNA polymerase II [12], cysteine plus methionine or glucagon, or of combinations of these. As shown in Figure 4 (upper panel), all agents caused a decrease in the mRNA

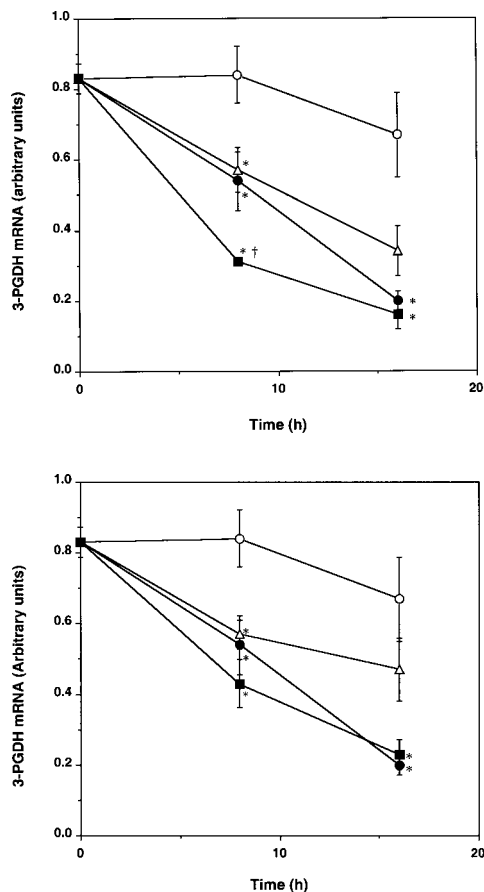


Figure 4 Effect of glucagon and cysteine plus methionine on the stability of 3-PGDH mRNA in rat hepatocytes

Hepatocytes, isolated from rats fed with laboratory chow, were precultured for 8 h in DMEM lacking cysteine and methionine and supplemented with 10% (v/v) fetal calf serum, 0.1 μM dexamethasone and 0.1 μM insulin. After attachment, the cells were washed once with PBS and the medium was replaced by serum-free medium and the culture was continued for 16 h. Then the medium was replaced by fresh medium and the culture was continued for the indicated durations in the absence (control, \circ) or in the presence of the following compounds: (upper panel) cysteine (140 μM) plus methionine (114 μM) (Δ), DRB (80 μM) (\bullet) or DRB plus cysteine plus methionine (\blacksquare); (lower panel) glucagon (1 μM) (Δ), DRB (\bullet) or DRB plus glucagon (\blacksquare). Total cellular RNA was extracted and analysed by Northern blotting. Results are means \pm S.E.M. for $n = 4$ values. *Significantly different ($P < 0.05$) from control by Student's t test; \dagger significantly different from DRB alone.

concentration; the effect of cysteine plus methionine was, however, additive with that of DRB at 8 h but not at 16 h. In contrast, no significant additivity was observed when glucagon was combined with DRB (Figure 4, lower panel).

Effect on transcription

Several attempts were made to measure the effect of glucagon, insulin and cysteine plus methionine on the rate of transcription of the 3-PGDH gene by nuclear run-on assays. Nuclei were isolated from hepatocytes maintained for 16 h in serum-free DMEM lacking or supplemented with cysteine and methionine, in the absence or in the presence of insulin or glucagon. Very low levels of transcription of the 3-PGDH gene were observed in the five experiments that were performed (results not shown), preventing any precise quantitative analysis of the results, whereas fair signals, at least 30-fold stronger, were observed for the

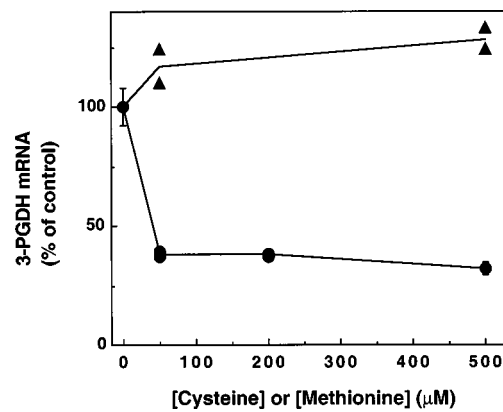


Figure 5 Effect of cysteine (\bullet) and methionine (\blacktriangle) on the level of 3-PGDH mRNA in rat hepatoma cells

FTO2B cells were cultured in DMEM/Ham's F12 to 70% confluence and then transferred to DMEM lacking serum, cysteine and methionine that was supplemented with the indicated concentrations of cysteine or methionine. After 24 h, RNA was isolated and analysed by Northern blot analysis. Results are means \pm S.E.M. for three values or individual values.

transcription of the β -actin gene. Comparison of the weak signals observed with the 3-PGDH probe suggested that transcription of 3-PGDH gene was lower in nuclei obtained from hepatocytes maintained in the presence of glucagon, whereas insulin or cysteine plus methionine were devoid of effect. The transcription of the β -actin gene was unaffected by glucagon.

Effect of cysteine and methionine on FTO2B hepatoma cells

Because hepatoma cells have a poor ability to synthesize cysteine from methionine [13], we postulated that they might be a suitable model for investigating the effects of cysteine and methionine separately. When FTO2B cells (rat liver hepatoma cells) that had grown to 70% confluence in DMEM/Ham's F12 medium were shifted for 24 h to DMEM containing no cysteine or methionine, there was a 5-fold increase in the 3-PGDH mRNA compared with cells maintained in complete medium (DMEM/Ham's F12). This increase was unaffected by supplementation of the medium with methionine but was markedly decreased if cysteine was added either without methionine (Figure 5) or with methionine (results not shown). In marked contrast with cysteine, neither glucagon (1 μM) nor dibutyryl-cAMP (0.5 mM), a derivative of its second messenger, affected the level of 3-PGDH mRNA in FTO2B cells, whether in the absence or in the presence of cysteine and methionine (results not shown).

DISCUSSION

Effect of protein starvation on amino acid concentration in plasma

The two amino acids whose concentrations changed most after prolonged protein starvation, although in opposite directions, were serine and cysteine. The increase in the concentration of serine, which was previously observed by Adibi et al. [14], presumably results from the induction of the serine pathway in the liver. It is indeed known that all three enzymes of this pathway are induced in this tissue by a protein-poor, carbohydrate-rich diet [1,15,16]. The marked relative decrease in the cysteine levels presumably reflects the fact that, besides protein synthesis, this amino acid fuels the formation of glutathione and sulphate, two compounds that can be irreversibly lost in detox-

ification reactions. The fact that the decrease in the cystine concentration was more important in animals fed with corn starch than in starved animals is most probably explained by the protein-sparing effect of carbohydrates, which results in a decrease in cysteine production by proteolysis [17,18].

Cysteine can be formed from serine and methionine; the induction of the serine pathway can be viewed as a mechanism favouring cysteine formation. However, this amino acid was in short supply in protein-starved rats despite the presence of a relatively elevated methionine concentration, possibly owing to low cystathionine synthase activity caused by the low-protein diet [19]. Cysteine was formed rapidly after the administration of a methionine load, which also resulted in a decrease in the serine levels. The fact that the cystine concentration was more elevated in this condition than after a cysteine load could be due to a difference in pharmacokinetics, cysteine being formed progressively from methionine.

Identity of the amino acid involved in the control of 3-PGDH gene expression

Protein refeeding results in a 50% decrease in 3-PGDH mRNA in 8 h and in its complete disappearance after 24 h. This effect can be mimicked by the intraperitoneal injection of a mixture of five amino acids: cysteine, methionine, tryptophan, threonine and valine. These amino acids were previously identified as having a role in the control of this enzyme's activity in the liver, in experiments in which rats were fed with different mixtures of essential amino acids with or without cystine [2]. Of these amino acids, cysteine and methionine were found in the present study to have the greatest ability to decrease the 3-PGDH mRNA, whereas threonine and tryptophan were much less active. This order of potency is similar to that observed by Mauron et al. [2].

Cystine was previously shown to substitute for methionine in the repression of 3-PGDH [1,2]. Because methionine can give rise to cysteine through the cystathionine pathway, this suggests that cysteine is the only active amino acid of these two. Two pieces of evidence indicate that this is so: (1) the concentration of cysteine in the plasma, and presumably its concentration in the liver, decreased more than that of methionine; (2) the effect of cysteine on the level of 3-PGDH mRNA, but not that of methionine, was also observed in hepatoma cells, known for their poor ability to form cysteine from methionine [13]. However, our results do not exclude the possibility that cysteine is not acting itself but has an effect through one of its metabolites.

Role of hormones

The fact that cysteine or its precursor methionine exerted effects of similar magnitudes *in vivo* and in cells in culture indicated that cysteine acts directly on the liver cells, i.e. without the intervention of a hormone or growth factor. However, cysteine or methionine were unable to cause the complete disappearance of the 3-PGDH mRNA both in cultured cells and *in vivo*, suggesting that another factor contributes to the effect of the mixture of amino acids in the intact animal. This factor is most probably not an amino acid, because the 3-PGDH mRNA was still expressed to approx. 20% of the induced level in hepatocytes incubated in the presence of cysteine and of all essential amino acids at concentrations higher than their concentration in plasma in control rats. Interestingly, glucagon also had an inhibitory effect on the expression of 3-PGDH mRNA, which was additive with that of cysteine. Glucagon is known to be secreted by α cells in response to several amino acids including tryptophan [20] and its concentration is more elevated in fasted rats than in carbohydrate-fed rats, whereas the converse is true for insulin [21]. It therefore

seems that the lack of expression of 3-PGDH in starvation probably results from the combined effect of a lower insulin concentration and increased glucagon and cystine concentrations in comparison with rats fed with corn starch.

Mechanism of the effects of cysteine and glucagon

That cysteine did not affect the rate of transcription of the 3-PGDH gene was indicated by the apparent lack of effect of this amino acid in nuclear run-on assays. This result is in keeping with the recent observation that cysteine does not affect the activity of the promoter of the 3-PGDH gene in transient transfection experiments in FTO2B cells (M. Robbi, Y. Achouri and E. Van Schaftingen, unpublished work). Furthermore, the fact that cysteine decreases the stability of the 3-PGDH mRNA indicates that it acts at a post-transcriptional level. The destabilization of the mRNA was observed at 8 h but not later, suggesting that the destabilization effect might be dependent on the presence of a factor that is rapidly degraded in the liver. The presence of such factor(s) might also explain why the mRNA decay did not follow first-order kinetics after the addition of the RNA polymerase inhibitor.

In contrast, nuclear run-on assays indicated that the effect of glucagon is exerted on the transcription of the gene. This is in agreement with the absence of a significant effect on mRNA stability. The fact that glucagon and cysteine act at different levels accounts easily for the additivity of their effects, which implies that the expression of 3-PGDH can be virtually turned on and off depending on the nutritional status.

The absence of effect of glucagon and of dibutyryl-cAMP in hepatoma cells contrasts with the effect of glucagon on hepatocytes and *in vivo*. This lack of effect has already been observed for other genes in hepatoma cell lines [22] and is most probably due to the difference in the pattern of gene expression when these cells are compared with fully differentiated hepatocytes.

Comparison with the regulation of other genes by amino acids

Other genes are known to be regulated by the protein content of the diet or *in vitro* by amino acids. Several genes have been studied in rat hepatoma cells and shown to be induced, repressed or unaffected by starvation of different essential amino acids. In some cases the regulation shows some degree of amino acid specificity [23]. It was shown recently in a human hepatoma cell line that the depletion of cysteine, arginine or any essential amino acids induces the expression of IGFBP-1 mRNA and protein [24].

Among enzymes involved in amino acid metabolism, asparagine synthetase is also known to be regulated by amino acid availability. In this case, the control is very different from that observed for 3-PGDH because it seems to be exerted by several amino acids and to occur at different levels, namely gene transcription, mRNA stability and translation [25–27].

Serine dehydratase, an enzyme involved in serine degradation, is reciprocally regulated in comparison with 3-PGDH, its activity being nearly undetectable in the livers of protein-starved rats and increasing more than 100-fold on a protein-rich diet [28]. As with 3-PGDH, the control seems to be exerted largely at the transcriptional level and to be partly effected by glucagon, which in this case stimulates the expression of the gene [29]. However, the administration of cysteine fails to stimulate the expression of this enzyme, in contrast with methionine, which increases the enzymic activity severalfold [1,2].

The two other enzymes of the serine biosynthesis pathway are also known to be induced by low-protein diets [1,16]. With phosphoserine phosphatase, cysteine was shown to be able to

inhibit the effect of the diet [1]. We therefore speculate that the regulation of gene expression by cysteine is a common characteristic of all three enzymes of the serine biosynthesis pathway. Owing to the large variations in its concentration, this amino acid represents an excellent candidate for a signal protein for malnutrition and it is likely that it is used to control other genes. This seems to be true of cysteine dioxygenase, the activity of which is increased severalfold after the administration of cysteine or methionine [30], for metallothionein, which also responds to the administration of cysteine [31], and for $\alpha 1$ type I collagen, whose expression in lung fibroblasts has been shown to be critically dependent on cysteine [32]. In this last case, it was shown that cysteine acts by increasing both transcription and mRNA stability.

We thank H.-G. Hers and F. Lemaigre for critical comments, and F. Vincent for help with the amino acid assays. This work was supported by Actions de Recherche Concertées, by the Belgian Federal Service for Scientific, Technical and Cultural Affairs and by Fonds National de la Recherche Scientifique.

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Received 30 April 1999/29 July 1999; accepted 31 August 1999