Evidence for a transient inhibitory effect of insulin on GLUT2 expression in the liver: studies *in vivo* and *in vitro*

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The glucose transporter GLUT2 is expressed predominantly in the liver. Previous studies have shown that glucose increases GLUT2 mRNA concentration in primary cultures of rat hepatocytes. Since insulin controls the glucose metabolism in the liver, it could be involved in the regulation of GLUT2 gene expression. *In vivo*, hyperinsulinaemia induced a transient inhibitory effect on liver GLUT2 gene expression, the maximal inhibition of GLUT2 mRNA concentration ($93 \pm 6\%$) being observed after 6 h. When hyperglycaemia was associated with hyperinsulin-

aemia, the decrease in liver GLUT2 mRNA concentration was partially prevented. The respective effects of glucose and insulin were studied *in vitro* by primary culture of rat hepatocytes. Insulin alone exerted a transient inhibitory effect on GLUT2 mRNA concentration. When insulin and glucose (10–20 mM) were associated, the stimulatory effect of glucose on GLUT2 gene expression was predominant. In conclusion, the present study shows that GLUT2 mRNA concentration was conversely regulated by insulin and glucose, both *in vitro* and *in vivo*.

INTRODUCTION

Glucose is a major source of energy for the mammalian cells. Glucose transport occurs via a facilitated diffusion mechanism mediated by a family of glycoproteins, the glucose transporters (GLUTs) [1]. These proteins, composed of at least five isoforms (GLUT1–5), share structural similarities but are expressed in a tissue-specific manner [1].

The first step of glucose metabolism in the liver is controlled by the glucose transporter GLUT2 [2–4]. GLUT2 mediates a bi-directional transport, insuring both entry and exit of glucose into and from the hepatocytes, depending on hormonal and metabolic conditions [2]. Although insulin does not acutely stimulate glucose transport in the liver [5,6], this hormone plays a crucial role in the control of several genes involved in hepatic glucose metabolism [7]. Indeed, the expression of phosphoenolpyruvate carboxykinase (PEPCK) (EC 4.1.1.32) and glucokinase (EC 2.7.1.1), key enzymes of liver gluconeogenesis and glycolysis, is under the control of insulin [4]. Insulin might also regulate GLUT2 expression in the liver.

A recent study has shown that liver GLUT2 mRNA concentration decreased in response to the early hyperinsulinaemia–hypoglycaemia induced by the destruction of pancreatic β -cells in the rat [8]. Furthermore, it has been previously reported that glucose increased GLUT2 mRNA concentration in cultured rat hepatocytes [9]. We have investigated whether insulin alone, or in association with glucose, could regulate liver GLUT2 expression. To study the specific effects of insulin and glucose on GLUT2 mRNA concentration, we performed euglycaemic– and hyperglycaemic–hyperinsulinaemic clamps and primary culture of rat hepatocytes. The expression of PEPCK and glucokinase was studied in parallel as controls of insulin action.

EXPERIMENTAL

Animals

Female Wistar rats (200 g) bred in our laboratory were housed at 24 °C with light from 07:00 to 19:00 h. They had free access to

water and chow pellets (65 % carbohydrate, 11 % fat and 24 % protein in terms of energy). Rats were anaesthetized with pentobarbital (50 mg/kg, intraperitoneally) and two catheters were inserted: one into the right jugular vein for blood sampling, and a second one into the left jugular vein for glucose, insulin or saline infusions. The rats were fasted for 24 h after implantation of the catheters. The glucose-clamp experiments were performed on conscious rats. A blood sample (500 μ l) was collected before the clamp experiments for determination of basal blood glucose and plasma insulin concentrations.

Control rats underwent pentobarbital anaesthesia and catheterization of jugular veins and were fasted for 24 h. They were infused with saline (0.9 % NaCl) for 3, 6 or 24 h. No statistical difference was observed in blood glucose or plasma insulin concentrations in control rats infused with saline for 3, 6 or 24 h (Table 1).

Euglycaemic– and hyperglycaemic–hyperinsulinaemic clamps

Human insulin (Actrapid; NOVO, Copenhagen, Denmark) was infused at a constant rate of 10 mmol/h per kg body wt. The blood glucose level was maintained by a variable infusion of glucose (30 %, w/v), which was started 1 min after the initiation of insulin infusion, and was set at 1.8 mmol/min per kg on the basis of previous experiments [10]. During the 3, 6 and 24 h euglycaemic–hyperinsulinaemic clamps, the coefficient of variation of blood glucose concentration was less than 16 % in each experimental group, and less than 12 % for the 6 h hyperglycaemic–hyperinsulinaemic clamps. In all the clamps, the plasma insulin concentration was raised to a mean value of 4 nM (Table 1). Blood samples (10 μ l) were taken every 5 min during the first 3 h, and then hourly until the end of the clamps. Glucose concentration was determined with a Glucose Analyser (model YSI 23A, from Yellow Springs Instrument Co., Yellow Springs, OH, U.S.A.). Additional samples (200 μ l) were taken hourly for plasma insulin determinations. The samples for insulin determination were immediately centrifuged at 4 °C, and the plasma was frozen at –20 °C. Plasma insulin was determined by

Abbreviation used: PEPCK, phosphoenolpyruvate carboxykinase.

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radioimmunoassay employing a commercial kit (Oris Industrie, Gif-sur-Yvette, France) using an antiserum raised in guinea pigs against human insulin. The antibody gives 100% cross-reaction with rat and human insulin. At the end of the clamp experiments the rats were killed, and the left liver lobule was removed and quickly frozen in liquid nitrogen and stored at -80°C .

Isolation of hepatocytes

Wistar rats weighing 200 g were used for hepatocyte isolation at 09:00 h, i.e. in the post-absorptive period. Hepatocytes were isolated by the method of Berry and Friend [11]. The dissociation of the cells was performed in Hepes buffer (NaCl 137 mM; KCl 2.7 mM; $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ 0.7 mM; Hepes 10 mM; pH 7.6, 37°C) containing 0.025% collagenase and 5 mM CaCl_2 . For each preparation used, the cell viability estimated by Trypan Blue exclusion was always greater than 90%.

Primary culture of hepatocytes

Isolated rat hepatocytes were suspended in Medium 199 containing Earle's salts, 2.2 mg/l NaHCO_3 (GIBCO BRL, Cergy-Pontoise, France) with penicillin (10 units/ml), streptomycin (100 $\mu\text{g}/\text{ml}$), kanamycin (50 $\mu\text{g}/\text{ml}$) and supplemented with fetal-calf serum (10%, v/v) (GIBCO BRL). Hepatocytes were plated in 100 mm plastic Petri dishes $[(5-6) \times 10^6 \text{ cells/dish}]$. After cell attachment (4 h) the medium was replaced by fresh Medium 199 containing 10% fetal-calf serum and various concentrations of glucose and insulin. We used 10% fetal-calf serum in the culture medium, since in preliminary experiments we showed that fetal-calf serum allowed us to maintain GLUT2 mRNA concentration at a level comparable with that observed in liver before cell dissociation for at least 48 h (results not shown). When the cultures were performed in the absence of glucose, the energy substrates lactate (10 mM) and pyruvate (1 mM) were added. Nevertheless, the glucose produced by the hepatocytes, and the presence of glucose in fetal-calf serum, led to a final glucose concentration of 1 mM in the culture medium. When the cultures were prolonged to 48 h, Medium 199 containing 10% fetal-calf serum and fresh insulin was replaced after 24 h.

Quantification of GLUT2, PEPCK and glucokinase mRNA concentrations

Total RNA was isolated from liver by the method of Chirgwin et al. [12]. The concentration of RNA was measured from the absorbance at 260 nm, and the RNA solutions were stored at -80°C . All samples had a A_{260}/A_{280} ratio of about 2.0. For Northern-blot analysis, 20 μg of RNA was denatured in a solution containing 2.2 mM formaldehyde and 50% (v/v) formamide by heating at 95°C for 2 min. They were size-fractionated by gel electrophoresis and transferred to a nylon membrane (Hybond N; Amersham International, Amersham, Bucks., UK). The GLUT2 cDNA probe was kindly supplied by Dr. B. Thorens [13], the PEPCK cDNA probe by Dr. R. W. Hanson [14], the glucokinase cDNA by Dr. P. Iynedjian [15] and the GLUT1 cDNA probe by Dr. G. I. Bell [16]. Probes were labelled with $[^{32}\text{P}]\text{-dCTP}$ by using the Multiprime labelling-system kit (Amersham). Hybridization was performed overnight at 42°C in a solution containing 42% deionized formamide, 7.5% dextran sulphate, $8 \times$ Denhardt's solution, 40 mM Tris/HCl, pH 7.5, and 1% SDS. The membranes were washed twice for 30 min in a solution containing $2 \times \text{SSC}$ ($\text{SSC} = 0.15 \text{ M NaCl}/15 \text{ mM sodium citrate}$) and 0.1% SDS at 42°C and twice for 30 min in

$0.1 \times \text{SSC}/0.1\% \text{ SDS}$ at 55°C . They were then exposed to X-ray film for 4–24 h at -80°C with intensifying screens. Quantifications were performed by scanning densitometry of the autoradiograms. The transferred RNAs were hybridized with an oligonucleotide specific for 18 S rRNA [17] labelled with $[^{32}\text{P}]\text{ATP}$ to control the amount of total RNA.

Quantification of GLUT2 protein

Liver membranes were prepared by the procedure described by Thorens and co-workers [13]. Membrane proteins (50 μg) were analysed by Western blotting [13], by using a polyclonal antibody directed against GLUT2 kindly provided by Dr. B. Thorens. The detection was performed with 0.2 $\mu\text{Ci}/\text{ml}$ ^{125}I -Protein A, followed by autoradiography.

Statistical analysis

Results are expressed as means $\pm$ S.E.M. Statistical analysis was performed by Students's *t* test for unpaired data.

RESULTS

Time course of insulin effects on liver GLUT2, PEPCK and glucokinase mRNA concentration *in vivo*

Euglycaemic-hyperinsulinaemic clamps were performed for 3, 6 and 24 h in order to assess the effect of hyperinsulinaemia on liver GLUT2 mRNA concentration *in vivo* (Figure 1). The three groups of rats had similar blood glucose and plasma insulin concentrations at the end of clamps (Table 1). Hyperinsulinaemia decreased GLUT2 mRNA concentration by $70 \pm 9\%$ after 3 h, and the maximal inhibition ($93 \pm 6\%$) was observed after 6 h (Figure 1). After 24 h of hyperinsulinaemia, liver GLUT2 mRNA concentration returned to the level detected in control rats (Figure 1), despite the maintenance of hyperinsulinaemia (Table 1). Liver GLUT1 mRNA concentration remained low and unchanged during euglycaemic-hyperinsulinaemic clamps (results not shown). The expression of PEPCK was markedly inhibited after 3, 6 and 24 h of hyperinsulinaemia (Figure 1). The maximal stimulatory effect of hyperinsulinaemia on glucokinase mRNA concentration was obtained after 3 h and was maintained at 6 and 24 h (Figure 1). The transient effect of insulin was thus specific to GLUT2 gene expression.

Figure 1 Time course of insulin effect of GLUT2, PEPCK and glucokinase mRNA concentrations *in vivo*

Euglycaemic-hyperinsulinaemic clamps were performed in 24 h-fasted rats. Hyperinsulinaemia was maintained for 3, 6 and 24 h. Values are means $\pm$ S.E.M. of 4–8 determinations: *significantly different ($P < 0.001$) from control values.

Table 1 Effects of euglycaemic–hyperinsulinaemic and hyperglycaemic–hyperinsulinaemic clamps on plasma glucose and insulin concentrations

Parameters	Saline infusions			Euglycaemic–hyperinsulinaemic clamps			Hyperglycaemic–hyperinsulinaemic clamps
	3 h (n = 3)	6 h (n = 4)	24 h (n = 8)	3 h (n = 3)	6 h (n = 4)	24 h (n = 8)	6 h (n = 4)
Glycaemia (mM)	4.2 ± 0.5	4.4 ± 0.4	4.3 ± 0.2	4.6 ± 0.5	4.3 ± 0.1	3.8 ± 0.2	12.7 ± 0.3*
Insulinaemia (nM)	0.20 ± 0.01	0.30 ± 0.01	0.30 ± 0.02	4.0 ± 0.1†	4.0 ± 0.4†	3.8 ± 0.4†	3.7 ± 0.2†

Results are means ± S.E.M.: * $P < 0.001$ compared with saline-infused rats and euglycaemic–hyperinsulinaemic rats; † $P < 0.001$ compared with saline-infused rats.

Figure 2 Dose–response effect of insulin on GLUT2 mRNA concentration in cultured hepatocytes

Isolated hepatocytes were cultured during 24 h in the presence of 5 mM glucose, 10% fetal-calf serum and various concentrations of insulin (0–100 nM). Representative Northern blots of GLUT2, PEPCK and GLUT1 are shown on the right side of the Figure. Values are means ± S.E.M. of 6 experiments: * significantly different ($P < 0.001$) from values measured in the absence of insulin.

Dose-dependent effect of insulin on GLUT2, GLUT1 and PEPCK mRNA concentrations in cultured hepatocytes

In order to determine whether the effect of insulin on liver GLUT2 gene expression was dose-dependent, we performed primary cultures of hepatocytes for 24 h in the presence of increasing concentrations of insulin (0–100 nM) and physiological glucose concentration (5 mM). The changes in PEPCK and GLUT1 mRNA concentrations were studied in parallel (Figure 2). Insulin inhibited GLUT2 gene expression in a dose-dependent fashion (Figure 2). The maximal inhibitory effect ($90 \pm 7\%$) was observed in the presence of 100 nM insulin, and the half-maximal effect was obtained at physiological insulin concentration (1–5 nM) (Figure 2). The insulin concentration necessary to obtain half-maximal inhibition of GLUT2 mRNA was similar to the insulin concentration reported to produce half-maximal inhibition of PEPCK expression [18]. GLUT1 mRNA concentration was not significantly modified whatever the insulin concentration in the culture medium (Figure 2).

Time course of the insulin effect on GLUT2 mRNA concentration in cultured hepatocytes

The time course of decrease in GLUT2 mRNA concentration by insulin was also studied in cultured hepatocytes. The maximal decrease in GLUT2 mRNA concentration in response to insulin

Figure 3 Time course of insulin effect on GLUT2 mRNA concentration *in vitro*

Primary culture of hepatocytes were performed during 48 h with 5 mM glucose, 10% fetal-calf serum and 100 nM insulin. Cells were harvested after 6, 18, 24 and 48 h. Values are means ± S.E.M. of 4 different experiments: * significantly different ($P < 0.001$) from control values (zero time).

was observed within 18 h of culture (Figure 3). *In vitro*, the inhibitory effect of insulin on GLUT2 mRNA occurred later (12 h) than *in vivo* (6 h; see Figure 1). After 48 h of culture, although fresh insulin was added in the culture medium after 24 h, GLUT2 mRNA concentrations was not significantly different from control values (Figure 3).

Effect of glucose concentration on GLUT2 and PEPCK mRNA levels in cultured hepatocytes

Cultures were performed for 24 h in the presence of various glucose concentrations (0–20 mM). Glucose increased GLUT2 mRNA concentration in a dose-dependent manner (Figures 4a and 4b). The maximal increase (4 ± 1 -fold) was observed in the presence of 20 mM glucose as compared with 0 mM glucose (Figure 4a). In the absence of insulin, the concentration of glucose necessary to induce a half-maximal increase in GLUT2 mRNA concentration was 4 mM (Figure 4a). Glucose concentration had no significant effect on PEPCK mRNA concentration (Figure 4b).

Combined effects of insulin and glucose on GLUT2 and PEPCK mRNA concentrations *in vitro* and *in vivo*

In vitro

The effect of glucose on GLUT2 gene expression was studied in the presence of 100 nM insulin, a concentration shown to induce maximal inhibition of GLUT2 (Figures 4a and 4b). In the absence or in the presence of glucose (5 mM), insulin significantly decreased GLUT2 gene expression (Figures 4a and 4b). The maximal inhibitory effect of insulin on GLUT2 ($90 \pm 7\%$) was

Figure 4 Effect of insulin and glucose on GLUT2 mRNA concentration in cultured hepatocytes

(a) Quantification of GLUT2 mRNA concentration by Northern-blot analysis. ○: Hepatocytes were cultured for 24 h in the absence of insulin and in the presence of various concentrations of glucose (0–20 mM); values are means $\pm$ S.E.M. of 5–10 experiments. The data are expressed as the percentage increase over the value at 5 mM glucose: * difference statistically significant ($P < 0.001$) compared with control values (5 mM glucose). ●: Hepatocytes cultured for 24 h in the presence of 100 nM insulin and various concentrations of glucose (0–20 mM); the values are expressed as percentages of the respective values obtained in the absence of insulin. Values are means $\pm$ S.E.M. of 3–7 experiments: † difference statistically significant ($P < 0.001$) when compared with the culture performed in the absence of insulin. (b) Representative Northern blot of GLUT2 and PEPCK mRNA concentrations in primary culture of hepatocytes. Hepatocytes were cultured for 24 h in the absence or in the presence of various glucose concentrations.

Figure 5 Effects of hyperglycaemia and hyperinsulinaemia on liver GLUT2 and PEPCK mRNA concentrations *in vivo*

(a) Representative Northern blot of liver RNA hybridized with GLUT2, PEPCK and 18 S rRNA probes; C, control rats infused during 6 h with 0.9% NaCl; EH, 6 h euglycaemic-hyperinsulinaemic rats; HH, 6 h hyperglycaemic-hyperinsulinaemic rats. (b) Quantification by scanning densitometry of autoradiograms from Northern-blot transfer analysis. Results are means $\pm$ S.E.M. of 4 different blots: * significantly different ($P < 0.05$) from control values; ** significantly different ($P < 0.001$) from control values.

Figure 6 Western-blot analysis of GLUT2 protein concentration in liver of control and euglycaemic-hyperinsulinaemic rats

A representative Western blot of GLUT2 is shown on which 50 μ g of protein of liver crude membranes per lane was loaded. Lanes: C, 24 h saline-infused rats; EH, 24 h euglycaemic-hyperinsulinaemic rats. Quantification by scanning densitometry of autoradiograms from Western-blot transfer analysis: results, expressed in densitometric arbitrary units, are means $\pm$ S.E.M. of 4 different blots.

	GLUT2 protein	Arbitrary units
	6 h	24 h
Control	100 $\pm$ 33%	99 $\pm$ 13%
Euglycaemic-hyperinsulinaemic clamp	130 $\pm$ 50%	108 $\pm$ 17%

observed at the physiological glucose concentration (5 mM). In the presence of high glucose concentrations (10 and 20 mM), the inhibitory effect of insulin was no longer present. Thus glucose exerted a predominant stimulatory effect on GLUT2 gene expression. In contrast, PEPCK gene expression was totally inhibited by insulin regardless of the glucose concentration present in the culture medium (Figure 4b).

In vivo

Hyperglycaemia was also able to prevent partially the inhibitory effect of insulin on liver GLUT2 expression *in vivo* (Figure 5). In hyperglycaemic-hyperinsulinaemic rats, liver GLUT2 mRNA concentration decreased by only 48 $\pm$ 2% as compared with control rats, whereas it decreased by 93 $\pm$ 6% in euglycaemic-hyperinsulinaemic rats (Figure 5). In contrast, PEPCK mRNA concentration similarly decreased in the liver of hyperglycaemic-hyperinsulinaemic and euglycaemic-hyperinsulinaemic rats (Figure 5).

Effect of insulin GLUT2 protein concentration *in vivo*

GLUT2 protein concentration was measured in order to check whether the changes in GLUT2 mRNA concentration could be accompanied by similar changes in protein levels. No significant change in GLUT2 protein levels was detected after 6 and 24 h of hyperinsulinaemia (Figure 6). Similarly, no significant change in GLUT2 protein levels was detected in hepatocytes cultured for 24 h in the presence of insulin (results not shown).

DISCUSSION

The respective roles of glucose and insulin on liver GLUT2 gene expression was investigated *in vivo* by using the glucose clamps and *in vitro* by using primary cultures of rat hepatocytes.

The regulation of liver GLUT2 expression has been studied previously in rats during fasting-refeeding and in diabetes. After 10 days of streptozotocin-induced diabetes, a slight increase in GLUT2 mRNA concentration was observed [19]. Fasting decreased and refeeding with carbohydrates increased liver GLUT2 mRNA concentration in the rat [20]. A recent study demonstrated

that liver GLUT2 mRNA concentration was transiently decreased in response to the hyperinsulinaemia-hypoglycaemia induced by the destruction of β -cells by streptozotocin [8]. The changes in liver GLUT2 gene expression in these situations could be due to several factors, including glycaemia, insulinaemia and/or counter-regulatory hormones. The euglycaemic clamp enabled us to study the effect of hyperinsulinaemia, independently of changes in glycaemia and counter-regulatory hormone concentrations. We showed that hyperinsulinaemia induced a rapid and transient decrease in GLUT2 mRNA concentration *in vivo*.

In order to study the respective roles of insulin and glucose on GLUT2 mRNA concentration, we performed experiments *in vitro*. Primary cultures of hepatocytes were chosen for the present study, although many hepatoma cell lines were available. Indeed, hepatoma cell lines (FAO, H4IIE) were not suitable for our purpose, because they expressed GLUT1, as do most transformed cell lines [21], far better than GLUT2 (results not shown). Insulin decreased GLUT2 mRNA concentration in cultured hepatocytes within 18 h. In a previous study, it was reported that the GLUT2 mRNA concentration was not decreased by 100 nM insulin in rat hepatocytes cultured for 20 h in the absence of glucose [9]. The difference between the results of the present study and those of Asano et al. [9], could be due to the fact that we used fetal-calf serum in the culture medium. The presence of fetal-calf serum during the entire culture period allowed us to maintain for 24 h an expression of GLUT2 comparable with that observed before cell plating (result not shown).

The effects of insulin on GLUT2 gene expression *in vivo* and *in vitro* were transient and disappeared despite the maintenance of hyperinsulinaemia. This suggests that the regulation of liver GLUT2 by insulin is rapidly down-regulated by an unknown mechanism. A transient effect of glucagon has been previously described for the PEPCK gene expression in cultured hepatocytes from adult rats. Indeed, PEPCK mRNA concentration was maximally induced by 2 h exposure to glucagon and then decreased to reach a basal value as the length of exposure to glucagon increased [18]. It was suggested that glucagon induced the synthesis of a destabilizing factor that could represent 'a self-regulated shut-off mechanism' of the glucagon-induced PEPCK gene activation [18]. Whether such an autoregulated factor could participate in the insulin inhibition of GLUT2 gene expression remains to be elucidated.

We showed that glucose induced a dose-dependent increase in GLUT2 mRNA concentration in cultured hepatocytes. This substantiates results from a previous study demonstrating that GLUT2 mRNA concentration was increased by glucose, mannose and fructose in cultured rat hepatocytes [9]. Whether this effect on GLUT2 gene expression is due to a direct effect of glucose or is due to one of its metabolites has yet to be established. Glucose has been shown to increase the transcription and the mRNA concentrations of several glycolytic or lipogenic-related enzymes such as aldolase B [22], L-pyruvate kinase [23] and spot S14 [24]. For spot S14 and L-pyruvate kinase, a *cis*-acting sequence called 'carbohydrate-responsive element' has been identified in the 5'-flanking region of the gene [25–27]. Many authors have postulated that a metabolite of glucose could be involved in the stimulation of gene transcription. Glucose 6-phosphate has been recently postulated as the glucose metabolite responsible for the accumulation of fatty acid synthase mRNA in cultured adipose tissue [28].

Furthermore, we showed that insulin and glucose exerted opposite actions on GLUT2 mRNA concentrations. When glucose and insulin were associated, both *in vivo* and *in vitro*, glucose prevented the inhibitory effect of insulin on GLUT2 gene expression. Indeed, in the presence of high glucose concentration

(10–20 mM), the inhibitory effect of insulin on GLUT2 gene expression was no more effective. It is rather unusual to observe an antagonistic effect of glucose and insulin on gene expression. The molecular mechanisms involved in the regulation of liver GLUT2 gene expression by insulin or glucose were not investigated in the present study, but could be located at the level of gene transcription and/or stabilization of mRNA concentration.

In euglycaemic-hyperinsulinaemic-clamp experiments, no change in GLUT2 protein levels was detected, despite the large decrease in GLUT2 mRNA. This could be explained by the transient nature of the inhibitory effect of insulin or by the fact that the half-life of GLUT2 protein is much longer than the half-life of GLUT2 mRNA. Since the rate of glucose transport is 50-fold higher than the rate of glucose phosphorylation [29], this suggests that GLUT2 is not the limiting step of glucose metabolism in the liver. Only a major decrease in the GLUT2 protein levels would have a regulatory influence on liver glucose utilization and production. This possibility cannot be excluded *a priori*, but such an effect was not observed in our experimental conditions.

In conclusion, we showed that GLUT2 mRNA concentration was conversely regulated by insulin and glucose both *in vivo* and *in vitro*: (1) physiological concentrations of insulin exerting a transient inhibitory effect on GLUT2; (2) glucose and insulin separately regulating GLUT2 in a dose-dependent manner; (3) the stimulatory effect of glucose (10–20 mM) on GLUT2 gene expression was predominant over the inhibitory effect of insulin.

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REFERENCES

- Bell, G. I., Kayano, T., Buse, J. B., Burant, C. F., Takeda, J., Lin, D., Fukumoto, H. and Seino, S. (1990) *Diabetes Care* **13**, 198–208
- Thorens, B., Charron, M. J. and Lodish, H. F. (1990) *Diabetes* **13**, 209–218
- Fukumoto, H., Seino, S., Imura, H., Seino, Y., Eddy, R. L., Fukushima, Y., Byers, M. G., Shows, T. B. and Bell, G. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5434–5438
- Granner, D. K. and Pilkis, S. (1990) *J. Biol. Chem.* **265**, 10173–10176
- Bauer, H. and Heldt, H. W. (1977) *Eur. J. Biochem.* **74**, 397–403
- Williams, T. F., Exton, J. H., Park, C. R. and Regen, D. M. (1968) *Am. J. Physiol.* **215**, 1200–1209
- O'Brien, R. M. and Granner, D. K. (1991) *Biochem. J.* **278**, 609–619
- Burcelin, R., Eddouks, M., Kandé, J., Assan, R. and Girard, J. (1992) *Biochem. J.* **288**, 675–679
- Asano, T., Katagiri, H., Tsukuda, K., Lin, J., Ishihara, H. and Oka, Y. (1992) *Diabetes* **41**, 22–25
- Leturque, A., Burnol, A.-F., Ferré, P. and Girard, J. (1984) *Am. J. Physiol.* **246**, E25–E31
- Berry, M. N. and Friend, D. S. (1969) *J. Cell Biol.* **43**, 606–620
- Chirgwin, J. M., Przybyla, A. E., McDonald, R. J. and Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299
- Thorens, B., Sarkar, H. K., Kaback, H. R. and Lodish, H. F. (1988) *Cell* **55**, 281–290
- Yoo-Warren, H. Y., Cimbala, M. A., Felz, K., Monahan, J. E., Leis, J. P. and Hanson, R. W. (1981) *J. Biol. Chem.* **256**, 10224–10227
- Iynedjian, P. B., UCLA, C. and Mach, B. (1987) *J. Biol. Chem.* **262**, 6032–6038
- Fukumoto, H., Seino, S., Imura, H., Seino, Y. and Bell, G. I. (1988) *Diabetes* **37**, 657–661
- Chan, Y.-L., Gutell, R., Noller, H. F. and Wool, I. G. (1984) *J. Biol. Chem.* **259**, 224–230
- Christ, B., Nath, A. and Jungermann, K. (1990) *Biol. Chem. Hoppe-Seyler* **371**, 395–402
- Oka, Y., Asano, T., Shibasaki, Y., Lin, Y., Tsukuda, K., Akanuma, Y. and Takaku, F. (1990) *Diabetes* **39**, 441–446
- Thorens, B., Flier, J. S., Lodish, H. F. and Kahn, B. B. (1990) *Diabetes* **39**, 712–719
- Gould, E. G. and Bell, G. I. (1990) *Trends Biochem. Sci.* **15**, 18–23
- Decaux, J. F., Antoine, B. and Kahn, A. (1989) *J. Biol. Chem.* **264**, 11584–11590
- Decaux, J. F., Marcillat, O., Pichard, A. L., Henry, J. and Kahn, A. (1991) *J. Biol. Chem.* **266**, 3432–3438
- Mariash, C. N., Seelig, S., Schwartz, H. L. and Oppenheimer, J. H. (1986) *J. Biol. Chem.* **261**, 9583–9586

- 25 Bergot, M. O., Diaz-Guerra, M. J. M., Puzeat, N., Raymondjean, M. and Kahn, A. (1992) *Nucleic Acids Res.* **20**, 1871–1878
- 26 Jacoby, D. B., Zilz, N. D. and Towle, H. C. (1989) *J. Biol. Chem.* **264**, 17623–17626
- 27 Thompson, K. S. and Towle, H. C. (1991) *J. Biol. Chem.* **266**, 8679–8682
- 28 Foufelle, F., Gouhot, B., Pegorier, J.-P., Perdureau, D., Girard, J. and Ferré, P. (1992) *J. Biol. Chem.* **267**, 20543–20547
- 29 Okuno, Y. and Gliemann, J. (1986) *Biochim. Biophys. Acta* **862**, 329–334
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