Mechanisms by which insulin, associated or not with glucose, may inhibit hepatic glucose production in the rat.

LUDOVIC GUIGNOT AND GILLES MITHIEUX
Institut National de la Santé et de la Recherche Médicale U.449,
Faculté de Médecine René Théophile Hyacinthe Laennec, F-69372 Lyon Cedex 08, France

Guignot, Ludovic, and Gilles Mithieux. Mechanisms by which insulin, associated or not with glucose, may inhibit hepatic glucose production in the rat. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E984–E989, 1999.—We investigated the intrahepatic mechanisms by which insulin, associated or not with hyperglycemia, may inhibit hepatic glucose production (HGP) in the rat. After a hyperinsulinemic euglycemic clamp in postabsorptive (PA) anesthetized rats, the 70% inhibition of HGP could be explained by a dramatic decrease in the glucose-6-phosphate (G-6-P) concentration, whereas the glucose-6-phosphatase (G-6-Pase) and glucokinase (GK) activities were unchanged. Under hyperinsulinemic hyperglycemic condition, the GK flux increased. The G-6-P concentration was not or only weakly decreased. The inhibition of HGP involved a significant 25% inhibition of the G-6-Pase activity. Under similar conditions in fasted rats, the GK flux was very low. The suppression of G-6-Pase and HGP did not occur, despite plasma insulin and glucose concentrations similar to those in PA rats. Therefore, 1) insulin suppresses HGP in euglycemia by solely decreasing the G-6-P concentration; 2) when combining both hyperinsulinemia and hyperglycemia, the suppression of HGP involves the inhibition of the G-6-Pase activity; and 3) a sustained glucose-phosphorylation flux might be a crucial determinant in the inhibition of G-6-Pase and of HGP.

Glucone-6-phosphatase: glucokinase: liver

GLUCOSE-6-PHOSPHATASE (G-6-Pase) catalyzes the last enzymatic reaction common to glycogenolysis and gluconeogenesis, e.g., the hydrolysis of glucose-6-phosphate (G-6-P) into glucose and Pi. It therefore confers on the liver the capacity to release glucose in blood. Glucokinase (GK) catalyzes the opposite reaction of phosphorylation of glucose. It therefore allows the liver to utilize glucose. Because of their strategic positions in the liver glucose metabolism, both of these enzymes are supposed to be the target of important regulatory mechanisms of hepatic glucose production (HGP; see Refs. 11 and 21 for recent reviews).

In agreement with the latter proposal, there has been abundant indirect evidence that the G-6-Pase activity might be attenuated during the postprandial period, via mechanisms involving either insulin (12, 19) and/or intrahepatic nutrient metabolites (5, 11, 17, 20, 21). Moreover, we recently demonstrated that the liver G-6-Pase activity is inhibited in rats during refeeding after a prior fasting period (18). On the other hand, the key role of GK in the regulation of HGP by insulin has been pointed out by studies in GK-deficient patients with maturity-onset diabetes of the young (8) and in transgenic mice with partial disruption of the GK gene (2).

Despite these advances, the mechanisms by which HGP is inhibited during the postprandial period remain largely unknown. This is even more true of the events taking place at the level of the G-6-Pase–GK cycle. The existence of posttranslational regulation mechanisms of G-6-Pase is a highly controversial issue because, with the exception of one in vivo study of refeeding in fasted rats (18), the attempts to demonstrate the inhibition of G-6-Pase activity under insulin infusion in euglycemia in rats or in isolated hepatocytes have repeatedly failed (12, 18, 19, 21). Because hyperglycemia per se is able to inhibit HGP, independently of insulin (24), we wanted to understand better the respective roles of insulin and glucose in the inhibition of HGP during the postprandial period in rats. We have paid special attention to G-6-Pase and GK and their respective substrates in these inhibition mechanisms of HGP.

MATERIALS AND METHODS

Animals and diets. Seven-week-old male Sprague–Dawley rats (IFFA CREDO, L'Arséle, France), weighing 220–240 g, were housed in the animal laboratory for at least 3 days and subjected to a standard light (0700–1900)-dark (1900–0700) cycle. Rats were fed with laboratory chow (50% carbohydrates, 23.5% proteins, 12% water, 5.5% mineral salts, 5% lipids, 4% cellulose; Unité Alimentation Rationnelle, Epinay-sur-Orge, France) and were given water ad libitum. Postabsorptive (PA) rats were deprived of food for 5 h with free access to water before each experiment. Fasted rats were deprived of food for 48 h with access to water.

Insulin and glucose infusions. Rats were anesthetized with a single intraperitoneal injection of pentobarbital (70 mg/kg body wt). A polyethylene catheter was inserted into the right jugular vein for infusion of insulin (Lilly France, Saint-Cloud, France) and/or glucose and [3-3H]glucose (specific activity 370 GBq/mmol; Isotopchim, Ganagobie, France) and were given water ad libitum. Postabsorptive (PA) rats were deprived of food for 5 h with free access to water before each experiment. Fasted rats were deprived of food for 48 h with access to water.

Insulin and glucose infusions. Rats were anesthetized with a single intraperitoneal injection of pentobarbital (70 mg/kg body wt). A polyethylene catheter was inserted into the right jugular vein for infusion of insulin (Lilly France, Saint-Cloud, France) and/or glucose and [3-3H]glucose (specific activity 370 GBq/mmol; Isotopchim, Ganagobie, France) and were given water ad libitum. Postabsorptive (PA) rats were deprived of food for 5 h with free access to water before each experiment. Fasted rats were deprived of food for 48 h with access to water.

Glucose-6-phosphatase: glucokinase: liver

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
glucose was infused at 2.4 ± 0.02 mmol/h. Body temperature was maintained at 37.5°C by a rectal probe-monitored blanket. After 180 min, a laparotomy was performed to allow free access to the liver. A liver lobe was frozen in situ with tongs cooled with liquid nitrogen (−196°C), weighed, and stored at −80°C. The rest of the liver was removed and weighed to determine the total liver weight and to isolate microsomes according to previously published procedure (17–19). Blood was collected for the determination of plasma glucose and insulin concentrations and of specific activity of glucose. This protocol was approved by a local ethics committee for animal experimentation.

Enzyme and metabolite assays. G-6-Pase was assayed at 37°C and pH 7.3 in liver homogenates obtained from freeze-clamped samples and in isolated microsomes as described previously (17, 18). The contribution of nonspecific phosphatase activity was estimated via the hydrolysis of β-glycerophosphate under the same conditions of assay and was subtracted from all measurements. GK was assayed at 37°C and pH 7.3 in 12,000 g supernatants of liver homogenates as described by Bontemps et al. (7). The results are expressed as enzymatic unit; one unit is the amount of enzyme that converts one micromole of substrate per minute under the conditions of the assay. G-6-P concentration was determined as described by Lang and Michal (14).

Western blot analyses. A rabbit polyclonal antiserum against the G-6-Pase catalytic unit was obtained by injection of a synthetic peptide matching the 14 amino acids of the COOH-terminal end of the G-6-Pase sequence (CLARLGGQTHKKSL), coupled to keyhole limpet hemocyanin. Protein content of liver homogenates or microsomes was measured by the Bradford procedure (Bio-Rad, Ivry-sur-Seine, France) with bovine serum albumin as a standard. Proteins (25 mg) were subjected to electrophoresis on 9% polyacrylamide gels in the presence of SDS and electrophoretically transferred to immunoblot-polyvinylidene difluoride membranes (Millipore, St Quentin-sur-Yvelines, France). After being blocked, the membranes were incubated with a 1/2,000 dilution of anti-G-6-Pase antiserum followed by a 1:10,000 dilution of goat anti-rabbit IgG fraction conjugated with peroxidase (Sigma, La Verpillière, France). The detection was performed using a chemiluminescence-hyperfilms system (Speci Ste Feye-les-Lyon, France) with enhanced chemiluminescence-hyperfilms (Amersham, Les Ulis, France).

Other methods and calculations. Plasma glucose concentration was determined by the method of Bergmeyer et al. (4). Plasma immunoreactive insulin concentration was assayed by radioimmunoassay using a kit INSIK-5 (Sorin-Biomedica, Saluggia, Italy) with human insulin as a standard (100% cross-reactivity with rat insulin). HGP was assessed by radioimmunoassay using a kit INSIK-5 (Sorin-Biomedica, Saluggia, Italy) with enhanced chemiluminescence-hyperfilms (Amersham, Les Ulis, France), coupled to keyhole limpet hemocyanin. Protein content of liver homogenates or microsomes was measured by the Bradford procedure (Bio-Rad, Ivry-sur-Seine, France) with bovine serum albumin as a standard. Proteins (25 mg) were subjected to electrophoresis on 9% polyacrylamide gels in the presence of SDS and electrophoretically transferred to immunoblot-polyvinylidene difluoride membranes (Millipore, St Quentin-sur-Yvelines, France). After being blocked, the membranes were incubated with a 1/2,000 dilution of anti-G-6-Pase antiserum followed by a 1:10,000 dilution of goat anti-rabbit IgG fraction conjugated with peroxidase (Sigma, La Verpillière, France). The detection was performed using a chemiluminescence-hyperfilms system (Speci Ste Feye-les-Lyon, France) with enhanced chemiluminescence-hyperfilms (Amersham, Les Ulis, France).

Plasma glucose concentration was determined by the method of Bergmeyer et al. (4). Plasma immunoreactive insulin concentration was assayed by radioimmunoassay using a kit INSIK-5 (Sorin-Biomedica, Saluggia, Italy) with human insulin as a standard (100% cross-reactivity with rat insulin). HGP was assessed by radioimmunoassay using a kit INSIK-5 (Sorin-Biomedica, Saluggia, Italy) with enhanced chemiluminescence-hyperfilms (Amersham, Les Ulis, France).
Because extent as during our previous refeeding experiments in control saline perfused rats (Fig. 2), e.g., to the same 1.0 and 10.2 *6* 0.05 and 0.01, respectively). The key result in this case is that the G-6-Pase V$_{\text{max}}$ determined in microsomes isolated from a fresh liver lobe just after the removal of the freeze-clamped lobe. G-6-Pase indeed does not retain posttranslational regulations under these conditions (18, 19). G-6-Pase V$_{\text{max}}$ was not lower in liver microsomes isolated from glucose-infused rats (0.21 ± 0.02 and 0.21 ± 0.01 µmol·min$^{-1}$·mg protein$^{-1}$, respectively, means ± SE, n = 5) than in liver microsomes isolated from saline controls (0.19 ± 0.02 µmol·min$^{-1}$·mg protein$^{-1}$). These data strongly suggested that the decrease in the G-6-Pase V$_{\text{max}}$ induced by glucose infusion was dependent on a posttranslational mechanism of inhibition of the enzyme. On the other hand, there was no alteration in the G-6-Pase K$_{\text{m}}$ induced by glucose infusion. The G-6-Pase fluxes calculated from enzymatic parameters and G-6-P levels were thus significantly decreased in both groups of glucose-perfused rats compared with saline-perfused rats (57 ± 11 and 60 ± 5 vs. 100 ± 8 µmol·kg$^{-1}$·min$^{-1}$). Interestingly, CGP fitted HGP and was significantly attenuated on glucose infusion (Fig. 2).

Effect of glucose infusion in 48-h fasted rats. When compared with PA rats, control 48-h fasted rats (perfused with saline) exhibited a lower HGP (45 ± 2 µmol·kg$^{-1}$·min$^{-1}$), a lower G-6-P concentration (60 ± 8 nmol/g fresh liver), a much lower GK V$_{\text{max}}$ (0.3 ± 0.04 U/g fresh liver), and a higher G-6-Pase V$_{\text{max}}$ (23 ± 1.5 U/g fresh liver; Fig. 4). All data were expected and in agreement with previous published results (11, 12, 17, 18, 21). Glucose infusion induced a significant increase in the liver G-6-Pase activity and not to a decrease in the specific activity of the enzyme and not to a decrease in the amount of protein. The results of Fig. 3 strongly suggested that there was no alteration in the amount of G-6-Pase protein induced by the 3-h glucose infusion. Another estimate of the amount of G-6-Pase was the G-6-Pase V$_{\text{max}}$ determined in microsomes isolated from a fresh liver lobe just after the removal of the freeze-clamped lobe. G-6-Pase indeed does not retain posttranslational regulations under these conditions (18, 19). G-6-Pase V$_{\text{max}}$ was not lower in liver microsomes isolated from glucose-infused rats (0.21 ± 0.02 and 0.21 ± 0.01 µmol·min$^{-1}$·mg protein$^{-1}$, respectively, means ± SE, n = 5) than in liver microsomes isolated from saline controls (0.19 ± 0.02 µmol·min$^{-1}$·mg protein$^{-1}$). These data strongly suggested that the decrease in the G-6-Pase V$_{\text{max}}$ induced by glucose infusion was dependent on a posttranslational mechanism of inhibition of the enzyme. On the other hand, there was no alteration in the G-6-Pase K$_{\text{m}}$ induced by glucose infusion. The G-6-Pase fluxes calculated from enzymatic parameters and G-6-P levels were thus significantly decreased in both groups of glucose-perfused rats compared with saline-perfused rats (57 ± 11 and 60 ± 5 vs. 100 ± 8 µmol·kg$^{-1}$·min$^{-1}$). Interestingly, CGP fitted HGP and was significantly attenuated on glucose infusion (Fig. 2).

Effect of glucose infusion in PA rats. Glucose was infused at two different rates to obtain two different levels of hyperinsulinemia and hyperglycemia (Table 1). Under these conditions of elevated glycemia, the liver G-6-P concentration was not or only weakly decreased if any because differences were not statistically significant (Fig. 2). This could be ascribed to the increased GK fluxes (Fig. 2), as a consequence of increased glucose concentrations (Table 1), inasmuch as there was no modification of either GK V$_{\text{max}}$ or K$_{\text{m}}$ (not shown). The amount of glycogen was higher in the liver of glucose-infused rats (35.2 ± 6.1 and 47.1 ± 3.8 mg/g, low glucose and high glucose, respectively) than in the liver of saline-infused rats (13.3 ± 5.2 mg/g, P < 0.05 and P < 0.01, respectively). The key result in this case is that the G-6-Pase V$_{\text{max}}$ was decreased by 25% (10.5 ± 1.0 and 10.2 ± 0.6 vs. 13.7 ± 0.2 U/g fresh liver in control saline perfused rats; Fig. 2), e.g., to the same extent as during our previous refeeding experiments (18). Because V$_{\text{max}}$ was affected, we wished to ascertain that the inhibition was due to a decrease in the specific activity of the enzyme and not to a decrease in the amount of protein. The results of Fig. 3 strongly suggested that there was no alteration in the amount of G-6-Pase protein induced by the 3-h glucose infusion. Another estimate of the amount of G-6-Pase was the G-6-Pase V$_{\text{max}}$ determined in microsomes isolated from a fresh liver lobe just after the removal of the freeze-clamped lobe. G-6-Pase indeed does not retain posttranslational regulations under these conditions (18, 19). G-6-Pase V$_{\text{max}}$ was not lower in liver microsomes isolated from glucose-infused rats (0.21 ± 0.02 and 0.21 ± 0.01 µmol·min$^{-1}$·mg protein$^{-1}$, respectively, means ± SE, n = 5) than in liver microsomes isolated from saline controls (0.19 ± 0.02 µmol·min$^{-1}$·mg protein$^{-1}$). These data strongly suggested that the decrease in the G-6-Pase V$_{\text{max}}$ induced by glucose infusion was dependent on a posttranslational mechanism of inhibition of the enzyme. On the other hand, there was no alteration in the G-6-Pase K$_{\text{m}}$ induced by glucose infusion. The G-6-Pase fluxes calculated from enzymatic parameters and G-6-P levels were thus significantly decreased in both groups of glucose-perfused rats compared with saline-perfused rats (57 ± 11 and 60 ± 5 vs. 100 ± 8 µmol·kg$^{-1}$·min$^{-1}$). Interestingly, CGP fitted HGP and was significantly attenuated on glucose infusion (Fig. 2).

Effect of glucose infusion in 48-h fasted rats. When compared with PA rats, control 48-h fasted rats (perfused with saline) exhibited a lower HGP (45 ± 2 µmol·kg$^{-1}$·min$^{-1}$), a lower G-6-P concentration (60 ± 8 nmol/g fresh liver), a much lower GK V$_{\text{max}}$ (0.3 ± 0.04 U/g fresh liver), and a higher G-6-Pase V$_{\text{max}}$ (23 ± 1.5 U/g fresh liver; Fig. 4). All data were expected and in agreement with previous published results (11, 12, 17, 18, 21). Glucose infusion induced a significant increase in the liver G-6-Pase activity and not to a decrease in the specific activity of the enzyme and not to a decrease in the amount of protein. The results of Fig. 3 strongly suggested that there was no alteration in the amount of G-6-Pase protein induced by the 3-h glucose infusion. Another estimate of the amount of G-6-Pase was the
in both glycemia and insulinemia (Table 1). GK $V_{\text{max}}$ and $K_m$ were not significantly altered in glucose-perfused rats (data not shown). As expected, the amount of glycogen was lower in the liver of 48-h fasted rats infused with saline (1.5 ± 0.3 mg/g) than in the liver of PA rats under the same conditions (see Effect of insulin infusion in euglycemia in PA rats). It was significantly higher in the liver of 48-h fasted rats infused with glucose (7.0 ± 2.1 mg/g, P < 0.05 vs. saline). Because of the very low GK $V_{\text{max}}$, the GK substrate flux was very low compared with PA rats (compare Figs. 2 and 4), and despite the increased glycemia there was no or only a weak if any (not statistically significant) increase in the GK substrate flux induced by glucose infusion (Fig. 4). In strong agreement with a role for the GK flux in the inhibition of G-6-Pase activity, there was no inhibition of the G-6-Pase $V_{\text{max}}$ in fasted rats perfused with glucose (Fig. 4). In addition, there was no alteration in the G-6-Pase $K_m$ (not shown). The liver G-6-P concentration was not altered on glucose infusion. As a consequence of the latter data, the G-6-Pase flux was not modified. As calculated from these enzymatic data, the CGP was not decreased in glucose-perfused rats. This was in agreement with the absence of inhibition of HGP determined by tracer dilution technique (Fig. 4).

**DISCUSSION**

To understand better the respective roles of insulin and glucose in the inhibition of HGP during the post-prandial period, we first studied the effect of insulin infusion in euglycemia in PA rats. Under these conditions, insulin is able to inhibit HGP mainly by decreasing the G-6-P concentration. This might likely be accounted for by a stimulation of glycolysis, as previously suggested (1, 13, 19), and/or by an inhibition of the substrate flux from three-carbon precursors to G-6-P because insulin has no effect on the glycogen cycle under euglycemic conditions (1, 13, 19). In keeping with the latter, the liver glycogen content was the same in saline- and insulin-infused rats. It should be noted that such a strong decrease in the G-6-P concentration in the liver of PA rats is unlikely to occur under physiological conditions. A G-6-P concentration as low as that observed under insulin perfusion in PA rats (about 70 nmol/g of fresh liver) is indeed only observable in the liver of 48-h fasted rats in which the glucose phosphorylation flux is very low, due to both low glycemia and the near complete absence of GK (see Effect of glucose infusion in 48-h fasted rats and Ref. 17). Furthermore, there is no decrease in the liver G-6-P concentration during the postprandial period in rats (6 and C. Minassian and G. Mithieux, unpublished data).

Under conditions combining increased insulinemia and glycemia, the suppression of HGP appears to be the result of both the decrease in the G-6-Pase flux and the increase in the GK flux, e.g., the glucose-recycling rate. In this case, the decrease in the G-6-Pase flux involves the inhibition of the G-6-Pase activity, just as occurs during the postprandial situation in rats (18). It seems obvious that the increase in the liver glucose phosphorylation flux represents the major difference between the latter series of experiments, combining hyperinsulinemia and hyperglycemia, and the former one, with hyperinsulinemia in euglycemia. This strongly suggests that the GK flux could be the first step of the mechanism of inhibition of G-6-Pase. In agreement with this proposal, it has been reported that a partial deficiency in liver GK activity impairs the ability of hyperglycemia to suppress HGP in mice and rats (2, 3). We tested the hypothesis of a role for GK in the G-6-Pase inhibition induced by glucose perfusion in rats with near complete depletion in GK activity, e.g., long-term fasted rats (17). Despite increased glycemia and insulinemia, there was no inhibition of either the G-6-Pase $V_{\text{max}}$ or HGP in these animals.

Taken together, these results strongly support the idea that under comparable hyperinsulinemic and hyperglycemic conditions (see Table 1) a substantial
glucose-phosphorylating flux is a key factor in the G-6-Pase inhibition. Therefore, one might ask the question of the mechanism of the inhibition of G-6-Pase activity on refeeding in fasted rats (18) because rats used in this previous study were also fasted for 48 h before the refeeding experiments. In fact, the GK activity was higher by 2.5 times in the liver of fasted rats after refeeding for 3 h (about 0.7 U/g wet liver vs. 0.3 U/g (see Effect of glucose infusion in 48-h fasted rats, C. Minassian and G. Mithieux, unpublished data). This is in keeping with the rapid induction of liver GK activity during the feeding period in rats submitted to starvation-feeding cycles (10) and constitutes a marked difference with the experiments herein described involving glucose infusion, in which the GK activity is not significantly modified at the end of the infusion (see Effect of glucose infusion in 48-h fasted rats). Because of the increase of glycemia, the glucose-phosphorylating flux was thus expected to be significantly increased after 3-h refeeding in fasted rats. In agreement with the latter proposal, the liver G-6-P concentration was increased by almost 50% in 3-h refed rats compared with fasted rats (C. Minassian and G. Mithieux, unpublished data). The results previously obtained by refeeding in fasted rats (18) thus appear almost consistent with the important role of GK in the G-6-Pase inhibition mechanism demonstrated here.

It thus seems that insulin alone may inhibit HGP in euglycemia via the sole dramatic decrease in the liver G-6-P concentration. In contrast, under more physiological conditions combining both hyperinsulinemia and hyperglycemia, in which such a marked decrease in the hepatic G-6-P concentration does not occur, the inhibition of G-6-Pase activity is a required process for the suppression of HGP to take place. Additionally, our results strongly suggest that the glucose-phosphorylating flux is a crucial determinant in the G-6-Pase inhibition mechanism. Noteworthy, a very recent work in humans has also suggested that the suppression of HGP by glucose per se is dependent on an attenuation of the G-6-Pase flux (16). It would be very interesting to know whether hyperglycemia per se is able to induce the G-6-Pase inhibition in the absence of hyperinsulinemia. Therefore, we carried out a series of experiments involving saline or glucose infusions in PA rats in the presence of the powerful somatostatin analog octreotide. Unfortunately, octreotide infusion for 3 h (0.8 ng·min⁻¹·g⁻¹) induced a marked significant activation of the liver G-6-Pase Vₘₐₓ: 20.9 ± 2.2 U/g fresh liver vs. 13.7 ± 0.4 U/g in the control rats perfused with saline alone (means ± SE, n = 5, P < 0.01). This hindered a clear interpretation of the data in the rats perfused with glucose. Therefore, the question of whether hyperglycemia is able to induce the G-6-Pase inhibition mechanism in the absence of insulin remains unresolved.

Noteworthy, the requirement of hyperglycemia as a cofactor allowing the effect of insulin to take place has already been reported, relating to the effect of insulin on the liver glycogen metabolism in vivo. Insulin indeed stimulates the glycogen storage by activating glycogen synthase and attenuating glycogen phosphorylase under hyperglycemic conditions in rat, whereas it has no action on the same parameters under euglycemic conditions (1, 13, 19). The present study is in agreement with the latter because significant glycogen storage has occurred in rats in the presence of hyperglycemia and not in rats infused with insulin in euglycemia. The nature of the signal (G-6-P or another downstream glucose metabolite) required for insulin inhibition of G-6-Pase and of HGP to take place in the presence of hyperglycemia remains to be delineated. A possible candidate could have been xylulose 5-phosphate, an important metabolite of the pentose phosphate pathway, which has been suggested to mediate the effect of glucose on the gene expression of pyruvate kinase (9), phosphoenolpyruvate carboxykinase and G-6-Pase (15), and on the activity of fructose 6-phosphatase, 2-kinase/fructose-2,6-bisphosphatase (22).

However, the infusion of xylitol for 3 h in rats to increase the hepatic xylulose 5-phosphate concentration to the same extent as under hyperglycemic conditions induces an activation of G-6-Pase substrate flux and of HGP (15). It therefore seems that the activation of the carbon flux through the pentose phosphate pathway should not be the inducer of G-6-Pase inhibition and of HGP under hyperglycemic and hyperinsulinic conditions.

In conclusion, we have demonstrated that HGP may be suppressed in different ways by insulin, depending on whether hyperglycemia is concomitantly present. Insulin by itself is able to suppress HGP in euglycemia by solely inducing a marked decrease in the liver G-6-P concentration, likely because of its strong stimulating effect on glycolysis. In contrast, when such a marked decrease in the G-6-P concentration is prevented to occur because of concomitant hyperglycemia, the suppression of HGP involves both the increase of the GK flux (glucose-recycling rate) and the inhibition of the G-6-Pase activity. Even if one may not overlook insulin effects on other regulatory enzymes of gluconeogenesis, such as phosphoenolpyruvate carboxykinase (23), this work provides definitive evidence that posttranslational mechanisms of liver G-6-Pase constitute a crucial determinant in the regulation of HGP under conditions close to the physiological ones. Moreover, it has been strongly suggested that the inhibition of G-6-Pase activity, which takes place after refeeding in lean, fasted rats (18, 25), could be lost in genetically obese (fa/ fa) rats (25). This further points out the key role that the impairment of G-6-Pase regulatory mechanisms might play in the pathophysiological states associated with insulin resistance.

We greatly appreciated the help of Dr. R. Cohen for insulin determinations and of Dr. C. Minassian and of S. Montano for carrying out the experiments quoted in relation to refeeding in fasted rats.

Address for reprint requests and other correspondence: G. Mithieux, INSERM U.449, Faculté de Médecine R.T.H. Laennec, rue Guillaume Paradis, 69372 Lyon Cédex 08, France (E-mail: mithieux@laennec.univ-lyon1.fr).

Received 9 April 1999; accepted in final form 12 July 1999.
REFERENCES


