Activation of liver G-6-Pase in response to insulin-induced hypoglycemia or epinephrine infusion in the rat

ISABELLE BADY, CARINE ZITOUN, LUDOVIC GUIGNOT, AND GILLES MITHIEUX
Institut National de la Santé et de la Recherche Médicale U. 449, Faculté de Médecine Laennec, 69372 Lyon, France

Received 6 March 2001; accepted in final form 19 November 2001

Bady, Isabelle, Carine Zitoun, Ludovic Guignot, and Gilles Mithieux. Activation of liver G-6-Pase in response to insulin-induced hypoglycemia or epinephrine infusion in the rat. Am J Physiol Endocrinol Metab 282: E905–E910, 2002.—This study was conducted to test the hypothesis of the activation of glucose-6-phosphatase (G-6-Pase) in situations where the liver is supposed to sustain high glucose supply, such as during the counterregulatory response to hypoglycemia. Hypoglycemia was induced by insulin infusion in anesthetized rats. Despite hyperinsulinemia, endogenous glucose production (EGP), assessed by [3-3H]glucose tracer dilution, was paradoxically not suppressed in hypoglycemic rats. G-6-Pase activity, assayed in freeze-clamped liver lobes, was increased by 30% in hypoglycemia (P < 0.01 vs. saline-infused controls). Infusion of epinephrine (1 μg·kg⁻¹·min⁻¹) in normal rats induced a dramatic 80% increase in EGP and a 60% increase in G-6-Pase activity. In contrast, infusion of dexamethasone had no effect on these parameters. Similar insulin-induced hypoglycemia experiments performed in adrenalectomized rats did not induce any stimulation of G-6-Pase. Infusion of epinephrine in adrenalectomized rats restored a stimulation of G-6-Pase similar to that triggered by hypoglycemia in normal rats. These results strongly suggest that specific activatory mechanisms of G-6-Pase take place and contribute to EGP in situations where the latter is supposed to be sustained.

LIVER GLUCOSE-6-PHOSPHATASE (G-6-Pase) is a key enzyme in systemic glucose homeostasis, because it catalyzes the last biochemical reaction of glucose synthesis, i.e., the hydrolysis of glucose 6-phosphate (G-6-P) in glucose and phosphate (26, 29). It thus confers on the liver, the major gluconeogenic tissue, the capacity to release glucose into the blood to meet the glucose requirements of the body. In addition to regulation taking place at the level of gene expression, considerable evidence has recently been provided that regulatory mechanisms of the activity of G-6-Pase take place under conditions of inhibited endogenous glucose production (EGP) (see Refs. 26 and 29 for recent reviews). These particularly include suppressions of activity induced by insulin and/or glucose (6, 11, 12, 21, 25, 27) or nutrients that might be supplied upon refeeding (1, 5, 22, 23). On the other hand, in keeping with a crucial role of G-6-Pase under conditions of increased glucose requirements, it has been strongly suggested that G-6-Pase gene expression is rapidly induced to compensate for the glucose deficiency resulting from partial hepatectomy (13) or hemorrhage (17). Moreover, we have recently shown that the G-6-P hydrolytic flux is stimulated by glucagon infusion in vivo in the rat and in isolated hepatocytes and that this activation may account by itself for a concomitant increase in EGP (15). The mechanism in the latter case seems to involve temperature-sensitive (e.g., membrane-dependent) events rather than intrinsic stimulations of enzyme activity (15).

One crucial situation in which the liver is supposed to sustain a high glucose supply is hypoglycemia. This also constitutes a major problem that may be frequently encountered in the treatment of diabetic patients. In this work, we have thus raised the question whether G-6-Pase activity could be stimulated, contributing the production of glucose by the liver in response to insulin-induced hypoglycemia in rats. Additionally, we studied the involvement of counterregulatory processes in this phenomenon by: 1) testing the effect of infusion of dexamethasone (Dexa) or epinephrine (Epi) in normal rats; 2) performing the same experiments in adrenalectomized (ADX) rats. Because glucokinase (GK) and glucose 6-phosphate (G-6-P) are also crucial determinants of liver glucose production (12, 25, 29), we measured these two parameters in all experiments.

MATERIALS AND METHODS

Animals. Seven-week-old male Sprague-Dawley rats (220–240 g, IFFA-Credo, L’Arbresle, France) were housed for 1 wk of acclimatization in the laboratory and given standard chow (50% carbohydrate, 23.5% protein, 12% water, 5% lipid, 4% cellulose, 5.5% mineral salt, on weight basis) and water ad libitum. ADX rats (surgery performed at 4 wk of age) were further given sodium chloride (0.9%) and sucrose (1%) added in water. Experiments were performed in 8-wk-old animals in the postabsorptive state, i.e., 5 h after food withdrawal, with free access to drinking water.
Infusions. Rats were anesthetized by intraperitoneal injection of pentobarbital sodium (7 mg/100 g body wt). Polyethylene catheters were inserted into the right jugular vein for infusions and into the left carotid artery for blood sampling. [3-3H]glucose (Isotopchim, Ganagobie, France) was infused at 8.88 kBq/min to assess the glucose disappearance rate. A bolus (88.8 kBq/min) was infused for the first minute. Plasma glucose specific activity was not clamped, but it did not vary for at least the last 90 min of infusions (not shown). Insulin (Lilly France, St Cloud, France) was infused at 480 pmol/h for both euglycemic and hypoglycemic clamps (infusions were primed at 4.80 mmol/h for 1 min). A solution of glucose (1.67 mol/l) was infused to maintain either euglycemia (euglycemic clamps) or hypoglycemia (hypoglycemic clamps) above 3 mmol/l, as previously described (12, 23, 25). Dexa or Epi (Sigma, La Verpilliere, France) was infused in normal rats at 480 pmol/h for at least the last 90 min of infusions (not shown). Insulin infusion in EGP (Fig. 2) resulted in a dramatic suppression (77 ± 10 mmol·kg⁻¹·min⁻¹, means ± SE, n = 5) (Fig. 2A), despite plasma insulin levels being as markedly increased as those in euglycemic rats (Fig. 2F). G-6-Pase activity in hypoglycemic rats (11.2 ± 0.8 mmol·min⁻¹·g liver⁻¹) was higher by 40% (P < 0.01) compared with that in saline-infused rats, i.e., 8.1 ± 1.2 mmol·min⁻¹·g⁻¹ (Fig. 2B). However, there was no alteration in the G-6-Pase Michaelis-Menten constant (Km) induced by insulin either in euglycemia or in hypoglycemia (~2.5–3 mM). G-6-P content was higher by ~40% in the liver of insulin-infused hypoglycemic rats than in saline-infused euglycemic rats: 245 ± 19 vs. 175 ± 9 nmol/g liver (P < 0.05), respectively (Fig. 2C). GK activity was higher in insulin-infused hypoglycemic rats (2.0 ± 0.13 mmol·min⁻¹·g⁻¹) than in saline-infused rats (1.1 ± 0.02 mmol·min⁻¹·g⁻¹), whereas there was no change in GK activity induced by insulin infusion in the presence of euglycemia (Fig. 2D). GK Kms (~7–8 mM) were not different in both insulin-infused groups and in saline-infused rats.

The infusion of Dexa (0.7 μg·kg⁻¹·min⁻¹) for 180 min in normal rats had no significant effect on blood glucose (Fig. 1) or on any of the parameters studied but...
one (Fig. 2). Indeed, a slight lowering in GK activity was evidenced in Dexa compared with saline-infused rats: $0.8 \pm 0.04$ vs. $1.1 \pm 0.02 \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$, respectively ($P < 0.01$, Fig. 2D). In contrast, the infusion of Epi (1 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) induced a rapid and sustained increase in blood glucose (Fig. 1), a marked 80% enhancement in EGP ($138 \pm 13 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$, mean $\pm$ SE, n = 6), a 60% activation in G-6-Pase activity ($12.9 \pm 0.7 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ liver$^{-1}$), a 90% increase in the liver G-6-P content ($329 \pm 40 \text{mmol/g liver}$), compared with saline-infused controls (Fig. 2, A, B, and C). On the other hand, as in Dexa-infused rats, there was a slight decrease in the liver GK activity ($0.7 \pm 0.08 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$) in Epi-infused rats (Fig. 2D). There was no alteration in the G-6-Pase or GK $K_{\text{m},S}$ induced by either Dexa or Epi (not shown). Despite the dramatically increased plasma glucose concentration (Fig. 2E), there was no alteration of plasma insulin level in Epi-infused rats compared with saline controls (Fig. 2F).

Minute levels of Epi were found in the plasma of saline- and insulin-infused normal rats under conditions of euglycemia (compare with the levels in ADX rats in Table 1). There was a marked increase in plasma Epi induced by hypoglycemia, roughly equivalent to the levels reached during immobilization stress in the rat. Noteworthy, the plasma Epi concentration induced by Epi infusion was in the same order range (Table 1). In contrast, there was no increase in plasma norepinephrine promoted by insulin-induced hypoglycemia: $983 \pm 45$ vs. $781 \pm 64 \text{pg/ml}$ in saline-infused animals [not significant (NS)], in agreement with previous data (34). In the same manner, there was no difference in the plasma glucagon concentration in insulin-infused hypoglycemic rats compared with saline-infused control rats ($360 \pm 55$ vs. $306 \pm 25 \text{ng/l}$, respectively, NS). This was also in agreement with previous data (7). Because cortisol and/or Dexa has no effect on EGP (8, 10) or on the relevant liver metabolic parameters (see above), we further focused on the effect of insulin-induced hypoglycemia and of Epi in ADX rats.

**Effect of hormone infusions in ADX rats.** Basal EGP (saline-infused rats) was about twice lower in ADX-rats than in normal rats: $42 \pm 8$ vs. $77 \pm 10 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (P < 0.05), respectively (Fig. 3A). It had been necessary to infuse glucose in two of four rats to maintain euglycemia ($0.15 \pm 0.1 \text{mmol/h}$ as a mean). Insulin infusion in the presence of euglycemia (Fig. 1) induced a total suppression of EGP (Fig. 3A). However,

![Fig. 2. Effect of hormone infusions on plasma and liver glucose metabolic parameters in normal rats. Infusions in anesthetized rats were as in Fig. 1. EGP, endogenous glucose production; GK, glucokinase; G-6-Pase, glucose-6-phosphatase; G-6-P, glucose 6-phosphate. At the end of infusions (180 min), a liver lobe was frozen in situ and removed for enzyme and metabolite determinations, and blood was sampled for plasma hormone and glucose determinations. EGP was calculated from tracer data as described in MATERIALS AND METHODS. Results are expressed as means $\pm$ SE. *,$**$Different from saline, $P < 0.05$ and $P < 0.01$, respectively.]

| Table 1. Plasma epinephrine levels during infusions in normal and ADX rats |
|-----------------|-----------------|-----------------|
| **Infusions**   | **Normal Rats** | **ADX Rats**    |
| Saline          | 74 $\pm$ 11     | 132 $\pm$ 32    |
| Insulin (eu)    | 88 $\pm$ 21     | 89 $\pm$ 15     |
| Insulin (hypo)  | $5.221 \pm 1.022$* | $160 \pm 16$    |
| Epi             | $4.966 \pm 312$* | $7.520 \pm 71$* |

Data are expressed as means $\pm$ SE expressed in pg/ml. ADX, adrenalectomized; eu, euglycemic; hypo, hypoglycemic; Epi, epinephrine. *Significantly different from saline value, $P < 0.01$.
the amount of glucose infused to maintain euglycemia was not different from that in normal rats (1.39 ± 0.1 vs. 1.30 ± 0.2 mmol/h, see above). Insulin infusion to achieve hypoglycemia induced a rapid lowering of blood glucose (Fig. 1), resulting in a final plasma glucose concentration of 3.2 ± 0.2 mmol/l vs. 6.4 ± 0.7 mmol/l in saline-infused rats (P < 0.01, Fig. 3E). As in normal rats, there was no decrease in EGP in insulin-infused hypoglycemic ADX rats with regard to their euglycemic counterparts (Fig. 3A). However, it had been necessary to infuse glucose in three of four animals to maintain blood glucose above 3 mmol/l (0.3 ± 0.1 mmol/h as a mean). G-6-Pase activities and G-6-P levels were comparable in saline-infused ADX and normal rats (compare Fig. 2, B and C vs. Fig. 3, B and C). As in normal rats, insulin in euglycemia had no effect on G-6-Pase (Fig. 3B) and had a marked decreasing effect on the G-6-P content (Fig. 3C). In contrast with normal rats, there was no effect on G-6-Pase activity and G-6-P content induced by hypoglycemia in ADX rats (Fig. 3, B and C). GK activity was about two times lower in euglycemic ADX rats (0.5 ± 0.05 μmol·min⁻¹·g⁻¹) compared with euglycemic controls (P < 0.01). There was no stimulation of GK activity induced by hypoglycemia in ADX rats (Fig. 3D).

As expected, there was no increase in the plasma Epi concentration induced by hypoglycemia in ADX rats (Table 1). The infusion of Epi resulted in a marked increase in the plasma Epi level (Table 1). Noteworthy, the latter restored the effects induced by hypoglycemia in normal rats, e.g., rapid and sustained increase in blood and plasma glucose (Figs. 1 and 3E), marked increases in EGP (Fig. 3A), G-6-Pase activity (Fig. 3B), G-6-P content (Fig. 3C), and GK activity (Fig. 3D), and absence of increase in plasma insulin despite high glycemia (Fig. 3F).

Finally, it should be specified that in none of the experiments reported (relating to Figs. 2 or 3) might the increases in G-6-Pase activity be accounted for by any significant increase in the amounts of immunoreactive protein determined by Western blot (see, e.g., the results relating to Epi infusion in normal rats in Fig. 4).

**DISCUSSION**

The question whether biochemical mechanisms of regulation of G-6-Pase activity exist and participate in the modulation of EGP in vivo has been a matter of controversy for many years (14, 28). To date, we have provided a body of evidence that G-6-Pase activity is inhibited under conditions of suppression of EGP (6, 12, 23, 24), confirming and extending previous findings from other groups (11, 21). From those preceding studies, we have demonstrated that reliable conditions to characterize G-6-Pase activity biochemically under its regulation are available. Furthermore, the effects of hypoglycemia on EGP and G-6-Pase activity were restored by Epi infusion in ADX rats, demonstrating that the regulation of G-6-Pase activity by hypoglycemia is mediated through a neural mechanism involving Epi.

As noted above, in normal rats, Epi infusion resulted in a marked increase in plasma glucose (Fig. 1), accompanied by a marked increase in EGP (Fig. 3A) and a marked decrease in plasma insulin (Fig. 3F). These effects were restored in ADX rats after Epi infusion, indicating that the regulation of G-6-Pase activity by hypoglycemia is mediated through a neural mechanism involving Epi.

**Fig. 3.** Effect of hormone infusions on plasma and liver glucose metabolic parameters in adrenalectomized (ADX) rats. Infusions were as in Fig. 1 and plasma and liver determinations as in Fig. 2. Data are means ± SE; (significance as in Fig. 2).

![G-6-Pase Protein](http://ajpendo.physiology.org/)

**Fig. 4.** Effect of Epi infusion on the amount of immunoreactive G-6-Pase protein in normal rats. Twenty-five micrograms of freeze-clamped liver protein were analyzed in each track. Densitometric analysis did not reveal any significant difference between the two groups.
in insulin-induced hypoglycemic ADX rats. In this latter work, it was suggested that hyperinsulinemia and a high glucose-phosphorylating flux were both required to induce the inhibition of G-6-Pase activity in the liver of rats infused with glucose. Noteworthy, despite concomitant elevated plasma insulin concentration and enhanced glucose phosphorylation, G-6-Pase was activated in hypoglycemic rats. This suggested that another factor, independent of both the former factors and triggered by hypoglycemia (presumably counter-regulatory hormones), might be dominant over the effect of insulin and glucose phosphorylation, resulting in the activation of the enzyme.

Because glucagon and norepinephrine were not significantly increased in the plasma of hypoglycemic rats, in agreement with previous data (7, 34), and Dexa infusion had no effect on EGP and the relevant hepatic parameters (see RESULTS), the likely candidate hormone was Epi. In strong agreement with this hypothesis, plasma Epi was strongly increased in response to hypoglycemia. Furthermore, Epi infusion to establish plasma Epi levels very similar to those in hypoglycemic rats had very comparable effects, e.g., on the activation of G-6-P activity and increase in G-6-P content. Noteworthy, at slight variance with insulin-induced hypoglycemia, EGP and plasma glucose were additionally markedly increased in Epi-infused rats (see Fig. 2). This might likely be accounted for by the nonoccurrence of an increased liver glucose-recycling flux (no increase, even a decrease in GK activity). The latter could be explained by the absence of hyperinsulinemia as a result of the insulinoostatic effect of Epi (16). This absence of hyperinsulinemia and increased glucose-recycling flux and/or the presence of high plasma Epi levels might also explain why hyperglycemia is not able to inhibit EGP, at variance with previous data (12, 31). The experiments performed in ADX rats are in strong agreement with a crucial role of Epi in the G-6-Pase activation. It is beyond the scope of the present study to discuss the numerous metabolic alterations taking place in ADX rats. The key observation in these experiments has been that, despite very similar conditions of plasma insulin and glucose concentrations with regard to normal rats, no activation of G-6-Pase took place in insulin-induced hypoglycemic ADX rats (results of Fig. 3). It was noteworthy that Epi infusion not only induced a dramatic increase in EGP in ADX rats but also restored several events triggered by hypoglycemia in normal rats, e.g., a stimulation to a similar extent of G-6-Pase activity and the increase in G-6-P concentration, both contributing to the increased glucose production. It must be mentioned that Epi infusion likely altered blood parameters like pressure and heart rate. However, this could not alter the quantification of EGP, because only tracer and glucose infusions rates and glucose specific activity are required for the calculations in the one-pool model at steady state that we used (see MATERIALS AND METHODS).
In conclusion, several types of intracellular mechanisms may be crucially involved in the processes of control of EGFP in response to insulin-induced hypoglycemia, such as those that promote the increase in the liver G-6-P concentration, for example. Our results also strongly suggest a major role of Epi in these processes, in keeping with previous results (19, 20). However, the present work constitutes the first evidence that one of these mechanisms is the biochemical activation of the G-6-Pase enzyme. This definitively indicates that G-6-Pase and the molecular mechanisms of regulation of its activity constitute crucial factors involved in the control of EGFP, not only in the situations in which it is suppressed but also in those in which it has to be sustained.

We thank Drs. R. Cohen and J. M. Cottet-Emard for precious help in the insulin, glucagon, and catecholamine determinations.

REFERENCES
