

Portal Glucose Infusion in the Mouse Induces Hypoglycemia

Evidence That the Hepatoportal Glucose Sensor Stimulates Glucose Utilization

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To analyze the role of the murine hepatoportal glucose sensor in the control of whole-body glucose metabolism, we infused glucose at a rate corresponding to the endogenous glucose production rate through the portal vein of conscious mice (Po-mice) that were fasted for 6 h. Mice infused with glucose at the same rate through the femoral vein (Fe-mice) and mice infused with a saline solution (Sal-mice) were used as controls. In Po-mice, hypoglycemia progressively developed until glucose levels dropped to a nadir of 2.3 ± 0.1 mmol/l, whereas in Fe-mice, glycemia rapidly and transiently developed, and glucose levels increased to 7.7 ± 0.6 mmol/l before progressively returning to fasting glycemic levels. Plasma insulin levels were similar in both Po- and Fe-mice during and at the end of the infusion periods (21.2 ± 2.2 vs. 25.7 ± 0.9 μ U/ml, respectively, at 180 min of infusion). The whole-body glucose turnover rate was significantly higher in Po-mice than in Fe-mice (45.9 ± 3.8 vs. 37.7 ± 2.0 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively) and in Sal-mice (24.4 ± 1.8 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Somatostatin co-infusion with glucose in Po-mice prevented hypoglycemia without modifying the plasma insulin profile. Finally, tissue glucose clearance, which was determined after injecting ^{14}C -2-deoxyglucose, increased to a higher level in Po-mice versus Fe-mice in the heart, brown adipose tissue, and the soleus muscle. Our data show that stimulation of the hepatoportal glucose sensor induced hypoglycemia and increased glucose utilization by a combination of insulin-dependent and insulin-independent or -sensitizing mechanisms. Furthermore, activation of the glucose sensor and/or transmission of its signal to target tissues can be blocked by somatostatin. *Diabetes* 49:1635–1642, 2000

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Received for publication 2 November 1999 and accepted in revised form 26 June 2000.

ANOVA, analysis of variance; BAT, brown adipose tissue; ^{14}C -2DG, ^{14}C -2-deoxyglucose; Fe-mice, mice infused with glucose in the femoral vein; K_{ATP} , ATP-sensitive potassium channel; Po-mice, mice infused with glucose in the portal vein; Sal-mice, saline-infused mice; WAT, white adipose tissue.

Maintaining whole-body glucose homeostasis requires integration of several neural and hormonal signals activated by glucose-sensing systems located in different parts of the body. The glucose-sensing concept was originally proposed by Bernard (1). He suggested that the brain was able to sense hypoglycemia and trigger a counterregulatory response. More recently, glucose-sensitive neuronal elements have been found in the lateral and ventromedial hypothalamic nuclei (2,3), the nucleus of the solitary tract (4,5), and autonomic afferent nerves originating from visceral organs, such as the liver and the gastrointestinal tract (6–9). In particular, a glucose sensor has been localized in the portal vein upstream of the hepatic hilus (10). This hepatoportal glucose sensor has been shown to be connected through afferent hepatic branches of the vagus nerve (11) to glucose-sensitive neurons in the lateral hypothalamus (12,13) and in the nucleus of the solitary tract (14). The firing rate of these glucose-sensitive hepatic nerves is inversely proportional to the concentration of glucose infused in the portal vein (15,16). Activation of the hepatoportal sensor is, however, not simply responsive to elevation in glucose concentrations, but it requires the presence of a positive portoarterial glucose gradient (17–20). The hepatoportal glucose sensor has many physiological functions, including induction of anorexia (21,22), stimulation of hepatic glucose uptake (18–20,23), and inhibition of secretion of counterregulatory hormones induced by peripheral hypoglycemia (24–26).

In the present study, we analyzed the role of the mouse hepatoportal glucose sensor on the control of whole-body glucose utilization. We did so by infusing glucose into the portal vein at a rate equivalent to the net endogenous glucose production during the postabsorptive state. This infusion protocol induced a progressive paradoxical hypoglycemic state, which was associated with an increase in glucose utilization by oxidative muscles and brown adipose tissue (BAT). This correlation, however, could not be explained solely by a direct insulin effect. Furthermore, hypoglycemia could be prevented by somatostatin co-infusion with glucose by a

mechanism that did not involve any alteration in plasma insulin levels. We conclude that the hepatoportal glucose sensor plays a major role in stimulating glucose utilization by a subset of tissues and that its sensing function or the consequence of its activation can be impaired by somatostatin.

RESEARCH DESIGN AND METHODS

Surgical procedures. An indwelling catheter was implanted in mice into the vena cava through the left femoral vein. Precisely 10 mm of the catheter was inserted into the femoral vein reaching the vena cava. The other end of the catheter was slid under the back skin and exteriorized and glued at the back of the neck, as previously described (27–29). For portal glucose infusion, a second indwelling catheter was implanted into the portal vein through the splenic vein and glued at the back of the neck with the femoral catheter. To analyze the effects of chronic denervation on the soleus and the tibialis muscles, a segment of the left sciatic nerve was removed during the catheter implantation. This procedure altered the left leg motor activity. However, compared with nondenervated mice that underwent the operation, no change in feeding behavior was observed. This procedure was approved by the local animal care committee. When the mice were 10 to 14 weeks old, they were housed individually under an inverted dark-light cycle (8:00 A.M. to 8:00 P.M.). The mice were from our colonies and of a C57Bl/6 or C57Bl/6XSV129 background.

Infusion procedures. Four days after catheter implantations, food was removed at 8 A.M. for 6 h. Then, a 33% (weight/volume) glucose solution was infused in mice for 180 min through the femoral vein (Fe-mice) at a rate of 2 $\mu\text{l}/\text{min}$. Because the mice used in the study weighed between 27 and 33 g, the infusion rate ranged from 22.5 to 27.5 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. This rate is equivalent to the endogenous glucose production rate determined in mice fasted for 6 h (28). To establish a positive portoarterial glucose gradient, mice were infused with the glucose solution through the portal vein (Po-mice) at the same rate. To analyze the role of somatostatin (Somatostatin-14, Bachem, Bubendorf, Switzerland), the hormone was infused intravenously at a rate of 1 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ through the portal veins of the Po-mice.

To determine the rates of glucose turnover, whole-body glucose production, glycogenolysis, and glycogen synthesis, high-performance liquid chromatography-purified D-[3-³H]glucose (NEN LifeScience, Boston, MA) was infused continuously through the femoral vein at a rate of 10 $\mu\text{Ci} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. Plasma glucose, D-[3-³H]glucose, and ³H₂O concentrations were determined in 5 μl blood sampled from the tip of the tail vein every 20 min after the initiation of the glucose or the saline infusion (Fig. 1). A steady specific activity with variations <15% was obtained during the last hour of the infusion (Table 1). Mice showing larger variations in the specific activity were excluded from the study.

Glucose utilization index in individual tissues. To determine an index for the individual tissue glucose utilization rate, a flash injection of 1 μCi per gram of mouse of ¹⁴C-2-deoxyglucose (¹⁴C-2DG) (NEN LifeScience) through the femoral vein was performed 60 min before the end of the infusions (27,29–31). Plasma ¹⁴C-2DG disappearance was determined in 5- μl drops of blood sampled from the tip of the tail vein at 0, 5, 10, 15, 20, 25, 30, 45, and 60 min after the injection (Fig. 1).

Analytical procedures and determinations

Blood parameters. During infusions, blood glucose was determined every 10 min on a 3.5- μl drop of blood using a reflectance meter (Roche Diagnostic, Basel). Insulin was determined using an enzyme-linked immunosorbent assay kit (Crystal Chem, Chicago) before and at 10, 30, 50, 120, and 180 min after the beginning of the infusions in 5 μl plasma from 15 μl blood collected from the tail tip. Intra- and interassay coefficients of variation were 3.5 and 6.3%, respectively. At completion of the infusions, blood was collected, and plasma glucagon was assessed by radioimmunoassay (Linco, St Louis, MO). Intra- and interassay coefficients of variation were <5 and 15%, respectively.

Isotope measurements. ³H₂O, D-[3-³H]glucose, and ¹⁴C-2DG enrichments were determined from total blood after deproteinization. To perform the deproteinization, 5 μl venous blood from the tail was mixed with 250 μl of 0.3 mol/l ZnSO₄. Then, 250 μl of 0.3 mol/l Ba(OH)₂ was added to precipitate the proteins and blood cells (32). The formed Zn(OH)₂ precipitate was spun down. An aliquot of the supernatant was evaporated to dryness to determine the radioactivity corresponding to D-[3-³H]glucose. Another aliquot was directly mixed with the scintillation buffer to determine the radioactivity corresponding to ³H₂O and D-[3-³H]glucose. Therefore, the difference between the first and the second aliquot corresponds to the ³H₂O produced. In a third aliquot of the same supernatant, the glucose concentration was assessed by the glucose oxidase method (Trinder; Sigma). The ¹⁴C-2DG and ¹⁴C-2DG-6-phosphate tissue contents were determined as previously described (27,29,31). Briefly, a piece of each tissue was dissolved in 1 mol/l NaOH at 55°C for 60–120 min then neutralized with 1 mol/l hydrochloric acid.

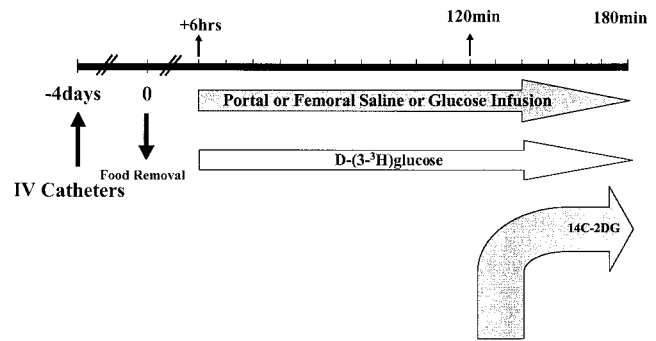


FIG. 1. Infusion procedures. At day –4, indwelling catheters were implanted into the portal vein and/or the left femoral vein. At day 0, food was removed for 6 h before the beginning of the infusions. Sodium chloride (0.9% saline) or glucose (25 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was infused through the femoral or the portal vein at a rate of 2 $\mu\text{l}/\text{min}$ for 3 h. D-[3-³H]glucose (10 $\mu\text{Ci} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was continuously infused through the femoral vein to assess the glucose turnover rate. Blood was sampled from the tail vein every 10 min to determine plasma glucose, ³H₂O, and D-[3-³H]glucose isotopic enrichments. To assess individual tissue glucose utilization, a flash injection of ¹⁴C-2DG (1 $\mu\text{Ci}/\text{g}$ body wt) was performed 60 min before the end of the infusions.

¹⁴C-2DG-6-phosphate and ¹⁴C-2DG were differentially precipitated by the use of a zinc hydroxide (0.3 mol/l) precipitation or a perchloric acid solution (6%) (30,32).

Calculations. Calculations were made with parameters collected during the last 60 min of the infusions when a steady-state D-[3-³H]glucose enrichment was obtained. Glucose turnover was calculated by dividing the D-[3-³H]glucose infusion rate by the plasma glucose-specific activity as previously described (27,29,31). The whole-body glycolysis rate was determined by dividing the rate of plasma ³H₂O accumulation per minute, which was linear during this period of time, by the plasma D-[3-³H]glucose-specific activity (33–35). Whole-body glycogen synthesis was calculated by subtracting the whole-body glycolytic rate from the glucose turnover rate. The means were calculated for each time point for this period. The mean value of mice from the same group was reported. Whole-body glucose clearance was calculated by dividing the glucose turnover rate by the blood glucose concentration.

Tissue glucose clearance was calculated by dividing the 2-deoxyglucose utilization rate (nanograms per milligram per minute) by the blood glucose concentration (milligrams per milliliter) at completion of the infusion period.

Statistical analysis. Results are presented as means \pm SE. Statistical significance of differences were analyzed by using Student's *t* test for unpaired bilaterally distributed values of equal variance. *P* < 0.05 was considered statistically significant. The statistical analysis of insulin and glycemic profiles during

TABLE 1
Mean D-[3-³H]glucose-specific activities during the last hour of the infusions

	Time (min)			
	120	140	160	180
Sal-mice	267,381	261,734	271,576	272,587
Po-mice	198,234	184,864	197,427	195,636
Fe-mice	284,764	302,252	292,043	284,414
Po-mice with somatostatin	278,959	293,306	280,025	276,558

Data are mean values of D-(3H-3)glucose specific activities for five to six mice per group. Plasma D-[3-³H]glucose enrichments were determined and divided by the plasma glucose concentration at the indicated time point to calculate the D-[3-³H]glucose-specific activity ($\text{cpm} \cdot \text{mg}^{-1} \cdot \text{ml}^{-1}$). Mice with variations of the glucose-specific activity >15% during the last hour of the infusions were not included in the study.

infusion experiments were performed by one-way analysis of variance (ANOVA) followed by Tukey's *t* test.

RESULTS

To evaluate the role of the murine hepatoportal glucose sensor on whole-body glucose homeostasis, we infused glucose into the portal vein of mice that were fasted for 6 h at a rate equivalent to hepatic glucose production ($\sim 25 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Figure 2A shows that this infusion protocol led to the development of hypoglycemia; glucose levels reached a nadir of $2.3 \pm 0.1 \text{ mmol/l}$ 180 min after the initiation of the glucose infusion and were maintained until the end of the infusion period. In a subset of mice, when glucose infusion was stopped, the blood glucose concentration returned to the fasting value within 10–20 min (data not shown). In saline-infused mice (Sal-mice), no hypoglycemia was induced, and the glycemic patterns were similar regardless of whether the infusions were through the portal or femoral vein. The data for both saline infusion protocols were then combined (Fig. 2A). As another control, glucose was infused through the femoral vein at the same rate as that through the portal vein. In the Fe-mice, hypoglycemia did not develop; rather, we observed transient hyperglycemia, which reached a maximum of $7.7 \pm 0.6 \text{ mmol/l}$ glucose 30 min after the initiation of the glucose infusion and then returned to normoglycemia.

In the three groups of mice, plasma insulin levels were then measured at the times indicated in Fig. 2B. In Po-mice, there was a slight increase in insulin levels (10.7 ± 0.6 to

$21.4 \pm 2.1 \text{ } \mu\text{U/ml}$), which occurred over the first 50 min and stayed at this level until the end of the infusion period ($21.2 \pm 2.2 \text{ } \mu\text{U/ml}$ at 180 min). In Fe-mice, insulin levels increased from 12.8 ± 1.2 to $28.3 \pm 2.1 \text{ } \mu\text{U/ml}$ over the first 30 min of the glucose infusion and remained stable until the end of the infusion period ($25.7 \pm 0.9 \text{ } \mu\text{U/ml}$ at 180 min). In Sal-mice, there was no increase in plasma insulin over the infusion period ($11.0 \pm 2.7 \text{ } \mu\text{U/ml}$ at 180 min).

Next, we determined if the hypoglycemia induced in Po-mice was due to an increase in whole-body glucose utilization. As shown in Fig. 3A, the glucose turnover rate increased to $45.9 \pm 3.8 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the Po-mice, a value significantly higher than that in both the Sal- ($24.4 \pm 1.8 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and the Fe-mice ($37.7 \pm 2.0 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Calculation of the glucose clearance rates increased these differences (Fig. 3B). Of the total glucose utilized, 65% was routed toward glycogen storage in Po-mice (Fig. 3C) and Fe-mice. Whole-body glycolysis was also significantly increased in Po-mice but not in Fe-mice.

Plasma glucagon concentrations were assessed at completion of the infusion period to analyze whether higher levels were associated with the hypoglycemic state. They were not increased in hypoglycemic Po-mice ($109 \pm 7 \text{ pg/ml}$) compared with Fe-mice ($110 \pm 10 \text{ pg/ml}$) or Sal-mice ($104 \pm 9 \text{ pg/ml}$).

Effect of somatostatin on the hepatoportal glucose sensor. Somatostatin is an inhibitor of insulin secretion by pancreatic β -cells. To determine if the stimulation of the hepatoportal glucose sensor was also under the control of somatostatin, this

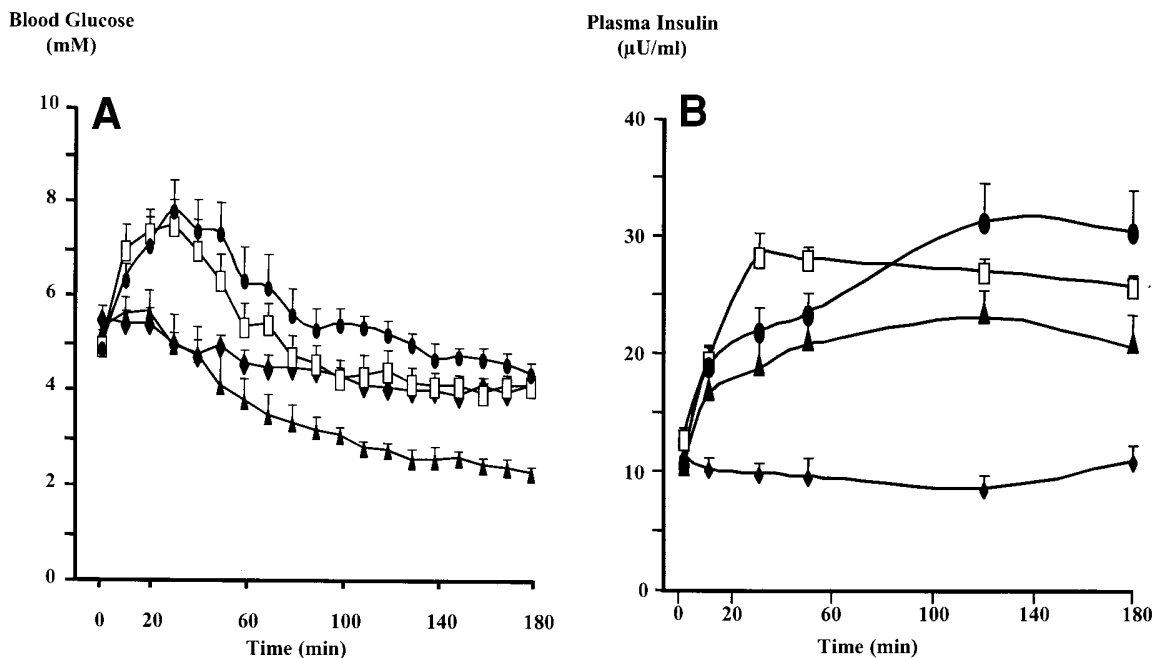


FIG. 2. Portal vein glucose infusion induces a paradoxical hypoglycemic state in mice. Glucose was infused through the portal or the femoral vein of mice at a rate of $25 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. In a subset of mice, somatostatin was co-infused with glucose through the portal vein, whereas other mice were infused with a saline solution. **A:** The glycemic pattern of the infused mice. Portal vein glucose infusion induced a paradoxical hypoglycemic state, whereas glycemia was stable in Sal-mice and characterized by a transient hyperglycemia in Fe-mice. Co-infusion of somatostatin with glucose at a rate of $1 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ prevented development of hypoglycemia in the Po-mice; instead, a transient hyperglycemia was observed. Each data point is the mean \pm SE for 12 mice per group. Glucose profiles of Po- versus Fe-mice and Po- versus Po-mice with somatostatin were statistically different by ANOVA ($P < 0.05$). **B:** Insulinemia during the glucose or saline perfusion protocols. Saline infusion did not induce an increase in plasma insulin levels. Insulin profiles of Po- versus Sal-mice, Fe- versus Sal-mice, and Po-mice with somatostatin versus Sal mice were statistically different by ANOVA ($P < 0.01$). Insulin profiles of Po-, Fe-, and Po-mice with somatostatin were not statistically different from each other. Data are means \pm SE for 5–6 mice per group. ♦, Sal-mice; □, Fe-mice; ▲, Po-mice; ●, Po-mice with somatostatin.

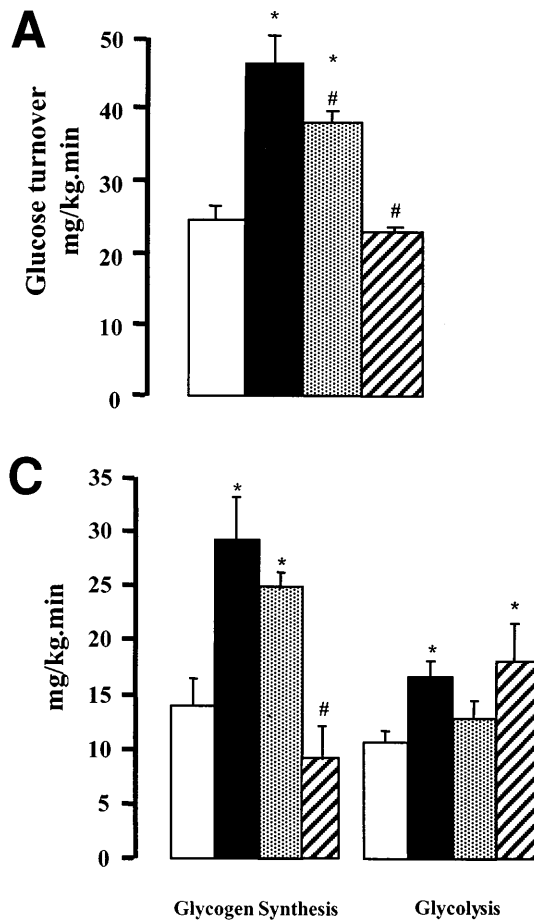


FIG. 3. Glucose utilization is increased by portal vein glucose infusion. **A:** The glucose turnover rate is increased to a higher level in Po-mice compared with Sal- and Fe-mice. Somatostatin co-infusion prevents this increase. **B:** Glucose clearance. Calculation of glucose clearance further emphasizes the difference in glucose turnover that is stimulated after activation of the hepatoportal glucose sensor. This increased clearance is, however, suppressed when somatostatin is co-infused with glucose through the portal vein. **C:** The increase in glucose utilized by the Po-mice is directed primarily (65%) to glycogen synthesis, but, compared with Sal-mice, there is also a significant increase in glycolysis. In Fe-mice versus Sal-mice, the increase in glucose utilization is due mainly to an increase in glycogen synthesis. Somatostatin co-infusion prevents the increase in whole-body glycogen synthesis. Data are means \pm SE for 12 mice per group. * $P < 0.05$ vs. Sal-mice. # $P < 0.05$ vs. Po-mice (without somatostatin). \square , Sal-mice; \blacksquare , Po-mice; \boxtimes , Fe-mice; \boxdot , Po-mice with somatostatin.

hormone was infused at a rate of $1 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. In preliminary studies, we determined that at completion of a 3-h hyperglycemic clamp (20 mmol/l blood glucose) in mice, the induced hyperinsulinemia was reduced but not completely suppressed by somatostatin co-infusion from ~ 500 to ~ 30 $\mu\text{U}/\text{ml}$. In the present study, co-infusion of somatostatin with glucose in the Po-mice completely suppressed the development of hypoglycemia (Fig. 2A). A transient hyperglycemia developed and was followed by a progressive return to fasted glycemia, a similar pattern to that observed in Fe-mice.

The effect of somatostatin on insulin levels in Po-mice was determined at the times indicated in Fig. 2B. No significant decreases in insulin levels were obtained by the somatostatin infusion in the Po-mice. The plasma glucagon concentration was 104.7 ± 14 pg/ml.

To determine whether the effect of somatostatin in preventing hypoglycemia development in Po-mice was attributable to an induced change in glucose utilization, we measured the whole-body glucose turnover rate. Figure 3A and B shows that, when somatostatin was co-infused with glucose in Po-mice, the increase in glucose turnover that was previously observed in the absence of somatostatin was suppressed and that the turnover and clearance rates were not different from that in Sal-mice.

The decrease in glucose utilization in Po-mice co-infused with somatostatin correlated with a decrease in whole-body glycogen synthesis (Fig. 3C).

Individual tissue glucose utilization and glucose clearance. Glucose utilization was assessed in several tissues to determine the contribution of individual tissues to the increase in whole-body glucose turnover rates. In addition, because the blood glucose levels were different in each group at the time of the glucose utilization measurement, the glucose clearance rate was calculated for each tissue. In the Po-mice compared with the Sal-mice, glucose utilization was unchanged in the brain, liver, epididymal fat (white adipose tissue [WAT]), and diaphragm (Fig. 4A), but glucose clearance rates were slightly increased (Fig. 4B). Conversely, in interscapular BAT and heart, glucose utilization and clearance rates were increased in Po- versus Fe-mice (Fig. 4A and B). These tissues are highly innervated and mainly oxidative for glucose. To analyze if the increase in glucose utilization was specific to these types of tissue, glucose utilization was also quantified in the soleus, a red oxidative muscle composed of slow-twitch fibers, and in the tibialis muscle, which is mainly composed of fast-twitch glycolytic fibers. Compared with Sal-mice, glucose utilization and clearance rates were unchanged in the tibialis of the Po-mice but were increased in the soleus muscle (Fig. 4A and B). Interestingly, this pattern was different from that of the Fe-mice, in which glucose utilization and clearance rates were stimulated in both the tibialis and the soleus. Because oxidative muscles such as the soleus are highly innervated and continuously contracting, the glucose utilization rate was determined in the soleus and the

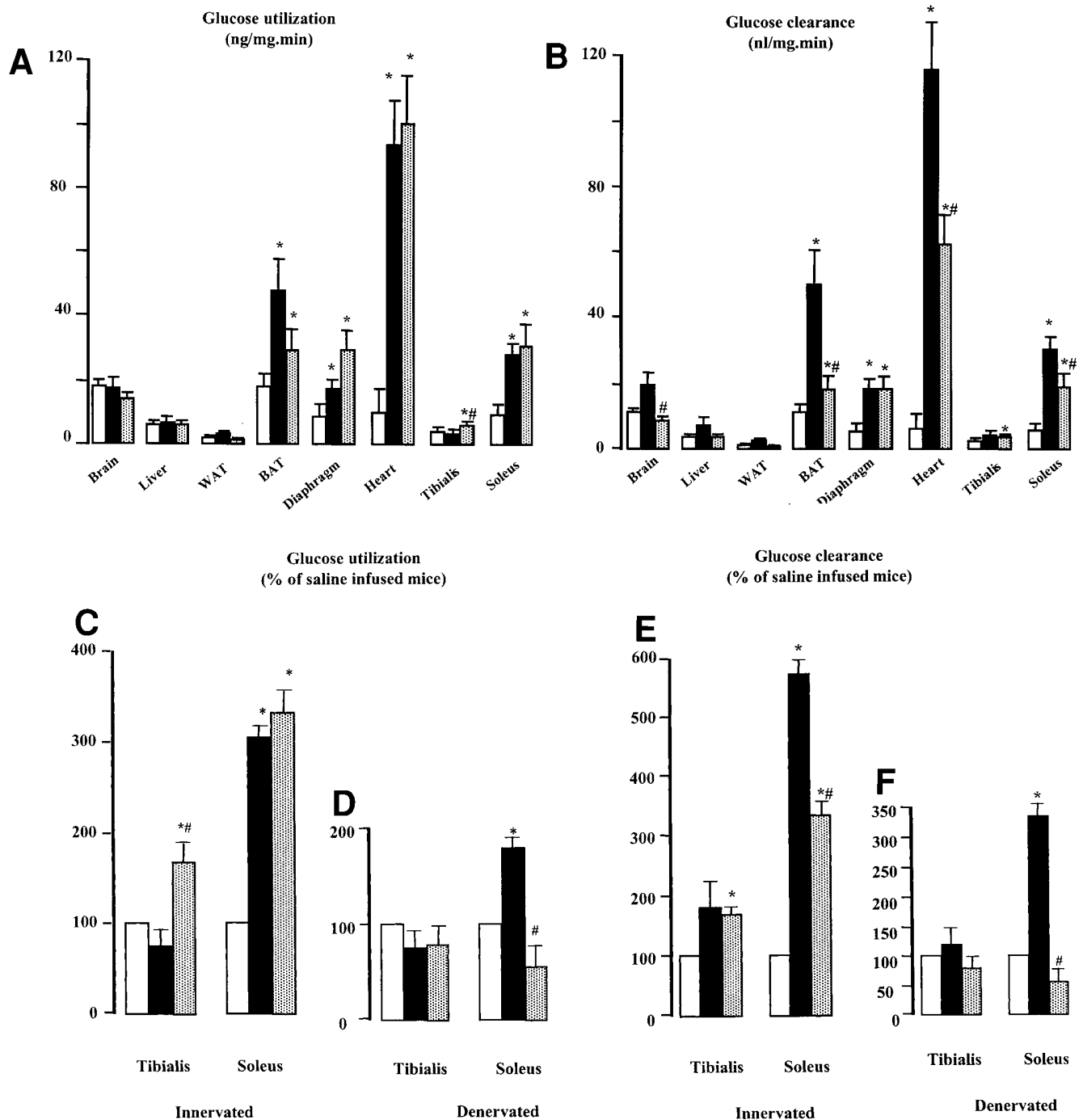


FIG. 4. Glucose utilization and clearance rates by individual tissues. **A:** A ^{14}C -2DG injection protocol was used to determine glucose utilization by individual tissues. Increased glucose utilization was observed in Po- and Fe-mice compared with Sal-mice in BAT, diaphragm, heart, tibialis, and soleus. **B:** Glucose clearance rates. The clearance rates were increased in Po-mice compared with Fe-mice in brain, BAT, heart, and soleus. **C and D:** Effect of denervation on glucose utilization by tibialis and soleus. The increase in glucose utilization in the soleus of the Po-mice was partly decreased by denervation, whereas it was completely suppressed in denervated soleus of Fe-mice. **E and F:** The effect of denervation on glucose clearance by the soleus muscle. Denervation reduced glucose clearance in the soleus of Po-mice by ~50%, whereas it completely suppressed it in the soleus of Fe-mice. Data are means \pm SE for 9–12 mice. □, Sal-mice; ■, Po-mice; ▣, Fe-mice. * $P < 0.05$ vs. Sal-mice; # $P < 0.05$ vs. Po-mice.

tibialis muscles after chronic denervation achieved by cutting the left sciatic nerve at the time of catheter implantation. As controls, the right soleus and tibialis muscles of the same mice were utilized. The increased glucose utilization rate by the

soleus of the Po-mice was only partly blunted by chronic denervation, whereas it was totally abolished in the Fe-mice (Fig. 4C and D). The calculation of the glucose clearance rate further increased these differences (Fig. 4E and F). In

Fe-mice, denervation of the tibialis also suppressed the glucose utilization and clearance rates (Fig. 4C–F).

DISCUSSION

The most striking observation of this study was that activation of the hepatoportal glucose sensor by glucose infusion led to a paradoxical hypoglycemic state. This state was accompanied by an increase in glucose utilization in a subset of tissues, and this effect could be blocked by secretion of somatostatin through a mechanism that does not involve a decrease in insulin secretion.

Development of hypoglycemia in Po-mice compared with Fe-mice was associated with an increase in whole-body glucose utilization. This increase probably resulted from a combination of insulin-dependent and -independent effects. The approximate twofold increase in insulinemia observed in Po-mice was similar to that seen in Fe-mice. In the presence of prevailing hypoglycemia, these levels of insulinemia certainly participated in the acceleration of glucose clearance. However, insulin cannot be the only factor that induces hypoglycemia, because in the presence of the same glucose infusion rate and a similar increase in insulinemia, Fe-mice did not develop hypoglycemia. Therefore, we must conclude that activation of the hepatoportal glucosensor also stimulates glucose utilization in peripheral tissues by an insulin-independent or an insulin-sensitizing effect.

Glucose levels were measured to further analyze the cause of hypoglycemia inducement. In Po- versus Fe-mice, whole-body glucose turnover was increased, and a greater part of the glucose concentration was directed toward glycogen synthesis. Analysis of the individual tissue glucose utilization rate using the ^{14}C -2DG injection protocol showed that clearance rates were higher in heart, soleus, and BAT of Po- versus Fe-mice. In contrast, glucose utilization by liver, WAT, or tibialis were similar in both glucose infusion protocols. These data suggest that the hepatoportal sensor of the mouse can preferentially stimulate glucose utilization by a subset of tissues.

The mechanism by which the portal signal is transmitted to target tissues to increase glucose utilization may involve activation of the autonomous nervous system. This hypothesis is supported by data indicating that the hepatoportal glucose sensor is connected to afferent branches of the vagus nerve, which modify the activity of neurons in different parts of the hypothalamus and the brainstem. In turn, these centrally located neurons could influence glucose disposal by peripheral tissues. The involvement of a gluconeuro-metabolic reflex is in agreement with several other studies. For instance, the role of the parasympathetic nervous system has been analyzed by the portal injection of the general muscarinic inhibitor atropine. This action induced insulin resistance and prevented glucose utilization in the hindlimb (36). Conversely, other studies showed that the electric stimulation of the autonomous nervous system increased skeletal muscle glucose utilization (36,37). On the other hand, direct stimulation of hypothalamic centers can modify peripheral glucose utilization. This has been shown by the direct intracerebroventricular administration of *N*-methyl-D-aspartate, which produced a rapid and sustained increase in whole-body glucose turnover (38) and individual skeletal muscle glucose utilization (39). In this latter study, glucose utilization was prevented when the skeletal muscles were denervated. We and others (29,40) have previously demonstrated that hypothal-

amic stimulation by leptin increased muscle glucose utilization through a neurally mediated and insulin-independent mechanism. In *ob/ob* mice (27), though, this is not the case, because the function of the autonomous nervous system is altered (41,42).

In our denervation experiments, glucose utilization in the soleus of Fe-mice was not increased over that of Sal-mice. This finding is consistent with the inducement of insulin resistance in denervated muscles. In the denervated soleus of Po-mice, however, glucose utilization was still increased, although it was 40% less than that in the innervated soleus. This discrepancy suggests that the stimulated glucose utilization in the soleus of Po-mice may be controlled either by a nervous connection between the hepatoportal sensor and the muscle that does not involve the sciatic nerve or by a non-neuronal signal, which may be a circulating factor. Others (43,44) have proposed that a hepatic factor could increase muscle glucose utilization.

One surprising feature of the Po-mice was that insulin levels increased when hypoglycemia developed and stayed elevated during the entire hypoglycemic period. Under these conditions, the insulin-secretory signal at the β -cell must be distinct from a glucose signal. Although we do not have a definitive explanation for this paradoxical stimulation of insulin secretion, it may originate from the hepatoportal glucose sensor by a pathway involving the autonomous nervous system.

Also, in the presence of hypoglycemia, elevated glucagon levels could be expected. However, data from the literature have shown that glucose activation of the hepatoportal glucose sensor can partly inhibit counterregulation induced by peripheral hypoglycemia. This effect was demonstrated by measuring a decrease in plasma catecholamine levels when glucose was infused in the portal vein of dogs, which were maintained in a hypoglycemic state by peripheral insulin infusions (10,24). Although we have not measured catecholamine levels in the present study, we determined that glucagon levels were identical in hypoglycemic Po-mice and in normoglycemic Fe- or Sal-mice at the completion of the infusions. Therefore, our data would suggest that counterregulation may be blunted in Po-mice, which would be in agreement with the observations of the literature previously reported. However, because we measured glucagon levels at the end of the infusion periods (180 min) only, we cannot rule out that a transient increase in plasma glucagon levels could have occurred earlier in the perfusion experiments. Nevertheless, in preliminary experiments, we maintained hypoglycemia (2.5 mmol/l) in mice for 3 h by insulin infusion (R.B., W.D., B.T., unpublished observations). These conditions induced an approximate twofold increase in glucagonemia compared with that in Sal-mice (168 ± 17 vs. 107.2 ± 9.2 pg/ml, insulin-infused vs. Sal-mice, respectively). When glucose was infused for 3 h through the portal vein and insulin was infused through the femoral vein to maintain hypoglycemia, no significant increase in plasma glucagon levels, compared with that in insulin-infused euglycemic mice, was observed (121 ± 9 and 101 ± 15 pg/ml, respectively). These data suggest that changes in glucagon secretion could be measured even after 3 h of infusion. Therefore, given the present conditions, an increase in circulating glucagon secretion during hypoglycemia was prevented by activating the portal glucose sensor.

One important aspect of our study is that infusion of somatostatin with glucose in Po-mice completely prevented the hypoglycemic state and actually led to a glycemic profile similar to that obtained in Fe-mice. This effect could not be explained by a suppression of insulin secretion, because the insulinemic pattern was similar in Po-mice in the presence or absence of somatostatin. Therefore, somatostatin could affect the hepatoportal sensor itself, the signal transmitted to the target tissues, or the target tissues directly. A possible direct effect of somatostatin on the hepatoportal glucose sensor is supported by the detection of somatostatin receptors in fiber arborizations with terminal nodular swellings located beneath the endothelium of the portal vein (45–47). Furthermore, it has been demonstrated that somatostatin increases the firing rate of the hepatic afferent vagus nerve (48), whereas glucose reduces it (49). Based on these data, and in accord with our findings, somatostatin is expected to have an antagonistic action toward glucose.

The finding that somatostatin infusion did not suppress insulin secretion is rather surprising, especially given our preliminary data showing that somatostatin infusion could dramatically reduce hyperinsulinemia during a hyperglycemic (20 mmol/l) clamp. The present experimental conditions are, however, very different. Indeed, during a hyperglycemic clamp, the presence of glucose is the signal that induces insulin secretion, and somatostatin has been described as an inhibitor of the ATP-sensitive potassium (K_{ATP}) channel-dependent glucose-signaling pathway (50,51). Furthermore, in the clamp experiments, insulinemia did not return to the fasted level of ~ 10 μ U/ml; rather, it returned to the fed level of ~ 30 μ U/ml. In the Po-mice, as previously discussed, there was an increase in insulin secretion from ~ 10 to ~ 30 μ U/ml, even though hypoglycemia was present, which indicated that β -cells were stimulated through a non-glucose-mediated signaling pathway. Therefore, the inability of somatostatin to block the observed increase in insulinemia may be because of the following three reasons: 1) because it may not inhibit insulin secretion induced by a non-glucose or K_{ATP} -independent signaling pathway, 2) because it is poorly effective in suppressing the low insulin-secretory activity induced by portal glucose infusion, and 3) because somatostatin, when infused into the portal vein, may be rapidly cleared as it passes through the liver. Whatever the exact mechanism, our data show a clear effect of somatostatin on the glycemic profile that cannot be explained by an impaired insulin secretion.

In this context, it is important to compare our data with those published by other researchers who have shown that the portal glucose signal decreased hindlimb glucose utilization (52,53). These studies not only used different animal models, such as the dog, but they also used a continuous somatostatin infusion to inhibit endogenous insulin and glucagon secretion, and these hormones were replaced by exogenous infusion. Thus, the differences between these results and our results may be due to the effect of somatostatin on the inhibition of the hepatoportal glucose-sensing pathway.

Activation of the glucoregulatory reflex originating from the stimulated hepatoportal glucose sensor may be an important part of the control of glucose homeostasis. In particular, in the absorptive period, when glucose enters the portal circulation, it can prepare peripheral tissues for the action of insulin and further minimize the glycemic excursion. The fact that glucose can induce hypoglycemia was already

reported almost 40 years ago. Indeed, in 1963, Russek (21,22) reported that rats that received an intraperitoneal glucose injection showed transient hypoglycemia 3 min later, an effect not observed after an intravenous glucose injection. These observations could be explained by an activation of the hepatoportal glucose sensor similar to that presented in this study. Intriguingly, one other possible manifestation of this glucose sensor may be revealed in the study of the muscle-specific insulin receptor knockout (MIRKO) mice (54). MIRKO mice do not have impaired glucose tolerance after an intraperitoneal glucose infusion, even though the insulin-signaling pathway in muscles, as well as the insulin-stimulated glucose uptake of isolated muscles, is strongly reduced. Our present data would suggest that normal glucose tolerance, despite impaired insulin-signaling in muscles, may be due to the stimulation of glucose utilization in peripheral tissues after activation of the hepatoportal glucose sensor, a situation similar to that described by Russek.

Together, our data show that stimulation of the hepatoportal glucose sensor leads to insulin-dependent and -independent stimulation of glucose utilization in a subset of tissues. The exact mechanisms by which the signal is generated and transmitted to target tissues and how it interacts with the insulin signal are presently unknown. However, one of the aims of this study was to establish an experimental protocol to test the functions of this glucose sensor in the mouse. Doing so will allow further studies of the molecular mechanisms of glucose detection and signal transduction by using mice with genetic inactivation of selected genes. The use of this experimental approach to evaluate the role of GLUT2 in the function of the hepatoportal glucose sensor is reported in the accompanying article (55).

ACKNOWLEDGMENTS

This work was supported by grants from the Swiss National Science Foundation (3100-055881.98/1 to R.B.) and (31-46958.96 to B.T) and from the Juvenile Diabetes Foundation International (1-1999-714 to R.B.).

We thank L. Tappy and W.F. Pralong for fruitful discussions.

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