

Glucose Sensing by the Hepatoportal Sensor Is GLUT2-Dependent

In Vivo Analysis in GLUT2-Null Mice

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In the preceding article, we demonstrated that activation of the hepatoportal glucose sensor led to a paradoxical development of hypoglycemia that was associated with increased glucose utilization by a subset of tissues. In this study, we tested whether GLUT2 plays a role in the portal glucose-sensing system that is similar to its involvement in pancreatic β -cells. Awake RIPGLUT1 \times GLUT2 $^{-/-}$ and control mice were infused with glucose through the portal (Po-) or the femoral (Fe-) vein for 3 h at a rate equivalent to the endogenous glucose production rate. Blood glucose and plasma insulin concentrations were continuously monitored. Glucose turnover, glycolysis, and glycogen synthesis rates were determined by the ^3H -glucose infusion technique. We showed that portal glucose infusion in RIPGLUT1 \times GLUT2 $^{-/-}$ mice did not induce the hypoglycemia observed in control mice but, in contrast, led to a transient hyperglycemic state followed by a return to normoglycemia; this glycaemic pattern was similar to that observed in control Fe-mice and RIPGLUT1 \times GLUT2 $^{-/-}$ Fe-mice. Plasma insulin profiles during the infusion period were similar in control and RIPGLUT1 \times GLUT2 $^{-/-}$ Po- and Fe-mice. The lack of hypoglycemia development in RIPGLUT1 \times GLUT2 $^{-/-}$ mice was not due to the absence of GLUT2 in the liver. Indeed, reexpression by transgenesis of this transporter in hepatocytes did not restore the development of hypoglycemia after initiating portal vein glucose infusion. In the absence of GLUT2, glucose turnover increased in Po-mice to the same extent as that in RIPGLUT1 \times GLUT2 $^{-/-}$ or control Fe-mice. Finally, co-infusion of somatostatin with glucose prevented development of hypoglycemia in control Po-mice, but it did not affect the glycemia or insulinemia of RIPGLUT1 \times GLUT2 $^{-/-}$ Po-mice. Together, our data demonstrate that GLUT2 is required for the function of the hepatoportal glucose sensor and that somatostatin could inhibit the glucose signal by interfering with GLUT2-expressing sensing units. *Diabetes* 49:1643-1648, 2000

Glucose-sensing units are present in different locations of the body, and their integrated functions participate in the adaptation of the organism to new metabolic situations. The hepatoportal glucose sensor can be activated by a portoarterial glucose gradient, and it controls numerous mechanisms, such as food intake (1-3), increased hepatic glucose uptake (4-7), inhibition of counterregulation (8-10), and increased glucose utilization by a subset of insulin-sensitive tissues (11). The molecules that control the function of this glucose sensor are still unknown. Molecules participating in glucose sensing have, however, been found elsewhere (e.g., the insulin-secreting pancreatic β -cells). In these cells, glucose induces insulin secretion by a signaling pathway, which depends on glucose uptake and metabolism (12). Uptake is controlled by GLUT2, and metabolism is initiated by phosphorylation by glucokinase. After subsequent metabolism through glycolysis and activation of oxidative phosphorylation, an increase in the ATP-to-ADP ratio triggers plasma membrane depolarization, influx of calcium, and insulin secretion.

The role of GLUT2 in glucose-stimulated insulin secretion has recently been established using islets from GLUT2 $^{-/-}$ mice (13). Perfusion experiments showed that in the absence of this transporter, there was a complete lack of first-phase secretion with, however, a preserved second phase. This reduced insulin-secretory response to glucose was the primary cause of death of the animals, which occurred between the second and third week of life, because insulin administration allowed survival of the animals to adulthood. Therefore, GLUT2 is essential for normal glucose sensing by pancreatic β -cells.

Here, we tested the hypothesis that GLUT2 also participates in the function of the hepatoportal glucose sensor. To perform these studies, we used GLUT2 $^{-/-}$ mice in which GLUT1 was reexpressed by transgenesis, specifically in the pancreatic β -cells. RIPGLUT1 \times GLUT2 $^{-/-}$ have a restored normal first phase of insulin secretion and live normally (14). As a readout for the signal generated by this sensor, we used the observation reported in the accompanying article (11) that glucose infusion through the portal vein induces hypoglycemia by a mechanism that involved increased glucose utilization in a subset of tissues, which could be blocked by somatostatin. In this study, hypoglycemia did not develop after portal glucose infusion in the absence of GLUT2. This finding correlated with an increase in glucose turnover, which was no longer

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ANOVA, analysis of variance; Fe-mice, mice infused with glucose through the femoral vein; GLP-1, glucagon-like peptide 1; Po-mice, mice infused with glucose through the portal vein; Sal-mice, saline-infused mice.

higher than that during glucose infusion in the femoral vein. Furthermore, somatostatin no longer affected the glycemic pattern or the glucose utilization of RIPGLUT1 \times GLUT2 $^{-/-}$ Po-mice. This development suggests that GLUT2 is involved in the function of the hepatoportal glucose sensor and that somatostatin could act on GLUT2-expressing cells.

RESEARCH DESIGN AND METHODS

GLUT2-null mice die between 2 and 3 weeks of age (13). Transgenic reexpression of GLUT1, specifically in pancreatic β -cells under the control of the rat insulin promoter (RIP), allows the mice to survive and breed (15). Therefore, the present studies were performed with RIPGLUT1 \times GLUT2 $^{-/-}$ mice at the age of 10–14 weeks. Control mice were of the RIPGLUT1 \times GLUT2 $^{+/+}$ genotype in a C57Bl/6 or C57Bl/6 \times SV129 background. The RIPGLUT1 \times GLUT2 $^{-/-}$ mice have restored normal first-phase glucose-stimulated insulin secretion, as assessed in perfusion experiments and hyperglycemic clamps (14).

Surgical procedures for catheter implantation, infusion procedures, and all analytical procedures for blood glucose parameters, isotope measurements, calculation of glucose turnover, whole-body glycogen synthesis, and glycolysis have been performed exactly as described in the accompanying article (11).

Results are presented as means \pm SE. Statistical significance of differences was analyzed by using Student's *t* test for unpaired bilaterally distributed values of equal variance. Values were considered different when $P < 0.05$. The statistical analysis of glycemic and insulinemic profiles during glucose infusions were performed by analysis of variance (ANOVA) followed by Tukey's *t* test.

RESULTS

Absence of hypoglycemia after portal glucose infusion in RIPGLUT2 $^{-/-}$ mice. To discern whether GLUT2 was involved in the hepatoportal glucose sensor, glucose was infused through the portal vein of control and RIPGLUT2 $^{-/-}$ mice (Po-mice) at a rate equivalent to the endogenous glucose production. In control mice, the portal vein glucose infusion induced a paradoxical hypoglycemic state (2.3 ± 0.1 mmol/l glucose) (Fig. 1A), which was not observed when glucose was infused through the femoral vein (Fe-mice), where transient hyperglycemia developed and was followed by a return to fasted glycemia (Fig. 1C). As described in the accompanying

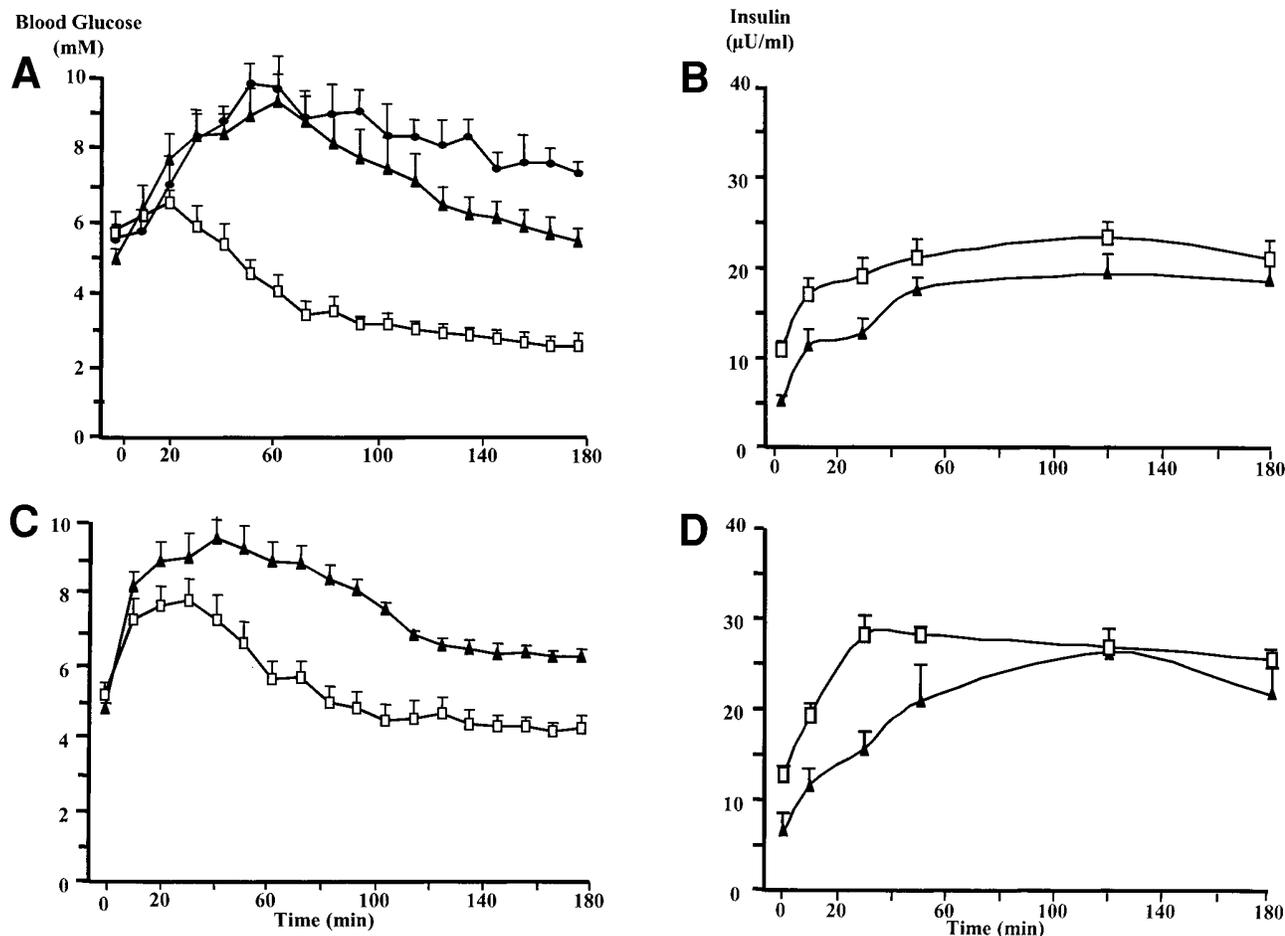


FIG. 1. Portal vein glucose infusion does not induce hypoglycemia in RIPGLUT1 \times GLUT2 $^{-/-}$ mice. **A:** Whereas portal vein glucose infusion induced hypoglycemia in control mice, transient hyperglycemia developed in RIPGLUT1 \times GLUT2 $^{-/-}$ mice. Reexpression of GLUT2 by transgenesis in hepatocytes (AATGLUT2 \times RIPGLUT1 \times GLUT2 $^{-/-}$ mice) did not restore the sensitivity to hypoglycemia development during portal glucose infusion. Glucose profiles of RIPGLUT1 \times GLUT2 $^{-/-}$ versus control mice were statistically different by ANOVA ($P < 0.05$). **B:** Measurement of the insulinemic profile over the period of portal vein glucose infusion. A slight increase in insulinemia was observed in both control and RIPGLUT1 \times GLUT2 $^{-/-}$ Po-mice but was not different by ANOVA. **C:** The glycemic patterns of control and RIPGLUT1 \times GLUT2 $^{-/-}$ Fe-mice showed no induction of hypoglycemia, but they did show transient hyperglycemia followed by the return to a fasted glycemic state. The increase in glycemia was higher and longer-lasting in RIPGLUT1 \times GLUT2 $^{-/-}$ mice and was statistically different ($P < 0.05$) from the profile of Fe-control mice. **D:** The insulinemic profiles over the period of femoral vein glucose infusion showed that insulinemia reached a similar level in control and RIPGLUT1 \times GLUT2 $^{-/-}$ mice, though with a small delay in the mutant mice. Insulinemic profiles were not different by ANOVA. Data are means \pm SE for 6–12 mice per group. \square , Control mice; \blacktriangle , RIPGLUT1 \times GLUT2 $^{-/-}$ mice; \bullet , AATGLUT2 \times RIPGLUT1 \times GLUT2 $^{-/-}$.

article (11), development of hypoglycemia in Po-mice compared with Fe-mice could not be explained by higher plasma insulin levels (Fig. 1C and D). In RIPGLUT1 \times GLUT2 $^{-/-}$ mice, the portal glucose infusion did not lead to hypoglycemia (Fig. 1A); rather, it led to transient hyperglycemia followed by a progressive return to fasted glycemia. This pattern was similar to that obtained when glucose was infused through the femoral vein of RIPGLUT1 \times GLUT2 $^{-/-}$ mice. The increases in insulinemia were slightly delayed in RIPGLUT1 \times GLUT2 $^{-/-}$ mice compared with control mice, but the insulinemic patterns were similar, regardless of the route of glucose infusion (Figs. 1B and D). Infusion of glucose through the femoral vein of control or mutant mice led to a significantly different glycemic pattern with a more pronounced hyperglycemic excursion in the RIPGLUT1 \times GLUT2 $^{-/-}$ mice (Fig. 1C). Infusion of a saline solution through the portal or femoral vein did not significantly increase or decrease the glycemia of control (11) or mutant mice (data not shown).

These initial experiments suggested that, in the absence of GLUT2, the hepatoportal glucose sensor was no longer functional. An alternative explanation could be that, in the absence of GLUT2 from the liver, the lack of hypoglycemia development is due to the suppression of glucose uptake by the liver. However, hepatic glucose uptake essentially occurs in the presence of hyperglycemia and hyperinsulinemia; thus, it should be negligible in the present situation. Nevertheless, to directly evaluate this possibility, we generated mice that reexpressed GLUT2 specifically in hepatocytes under the control of the α 1-antitrypsin (AAT) promoter. After appropriate breeding, we obtained AATGLUT2 \times RIPGLUT1 \times GLUT2 $^{-/-}$ mice, which express GLUT2 only in hepatocytes, as confirmed by Western blot analysis (16). These mice were infused with glucose into the portal vein using the same protocol used for the other mice. Figure 1A shows that reexpression of GLUT2 in hepatocytes did not restore glucose infusion-induced hypoglycemia. Therefore, the absence of GLUT2 from the glucose sensor system, not from hepatocytes, prevented normal inducement of hypoglycemia.

In control mice, portal glucose infusion increases whole-body glucose turnover to a greater extent than infusion of glucose through the femoral vein, which revealed that one of the functions of the activated hepatoportal glucose sensor was to stimulate whole-body glucose utilization (11). To further evaluate GLUT2's involvement in the function of the hepatoportal glucose sensor, we measured the rates of glucose turnover and whole-body glycolysis and glycogen synthesis in saline-infused (Sal-mice) or glucose-infused control and RIPGLUT1 \times GLUT2 $^{-/-}$ mice. Figure 2A shows that in control mice versus Sal-mice, glucose turnover was increased to a higher level by portal than by femoral glucose infusion. In contrast, in RIPGLUT1 \times GLUT2 $^{-/-}$ mice, glucose turnover was increased similarly by both portal and femoral infusion protocols and reached the same level as that in control Fe-mice. These differences between control and mutant mice were more striking when the clearance rates were calculated (Fig. 2B).

Whole-body glycolysis was then measured, and whole-body glycogen synthesis was calculated (Fig. 2C). The results showed a significant and similar increase in glycolysis in both control and RIPGLUT1 \times GLUT2 $^{-/-}$ Po-mice compared with Sal-mice. Whole-body glycogen synthesis was markedly stimulated in control but not in RIPGLUT1 \times GLUT2 $^{-/-}$ Po-mice versus Sal-mice. Together, these data indicate that in the

absence of GLUT2, glucose infusion through the portal vein did not generate the effect that normally stimulates glucose turnover in the control mice and activates glycogen synthesis. **Control of the hepatoportal glucose sensor by somatostatin.** We previously demonstrated that somatostatin could suppress development of hypoglycemia in control Po-mice without decreasing the plasma insulin levels. Here, we repeated these experiments in control and RIPGLUT1 \times GLUT2 $^{-/-}$ mice. Figure 3A shows that somatostatin co-infusion in control Po-mice suppressed the development of hypoglycemia and that the glycemic profile was similar to that of mutant mice that were co-infused with glucose and somatostatin in the portal vein. The glycemic pattern of the RIPGLUT1 \times GLUT2 $^{-/-}$ Po-mice was also similar in the presence and absence of somatostatin (Figs. 1A and 3A). The blood glucose profile was similar to that of control mice (Fig. 3A). Similarly, somatostatin did not modify the plasma insulin profile of the RIPGLUT1 \times GLUT2 $^{-/-}$ mice (Figs. 1B and 3B). However, insulin concentrations were lower in RIPGLUT1 \times GLUT2 $^{-/-}$ mice than in control mice. To determine whether the changes in glucose metabolism induced by activation of the portal sensor could also be inhibited by somatostatin, and to determine whether this inhibition involved a GLUT2-expressing unit, we performed the same glucose turnover measurements as previously described. It is shown in Fig. 2A and B that the increases in glucose turnover and glucose clearance induced by the portal glucose infusion were suppressed by somatostatin in control mice. In contrast, the increased glucose turnover observed in RIPGLUT1 \times GLUT2 $^{-/-}$ mice was not modified by somatostatin.

We then measured whole-body glycolysis and glycogen synthesis. Co-infusion of somatostatin in control Po-mice suppressed the significant increase in glycolysis observed during the absence of somatostatin (Fig. 2C) and glycogen synthesis. In RIPGLUT1 \times GLUT2 $^{-/-}$ mice, somatostatin co-infusion did not affect glycolysis or glycogen synthesis (Fig. 2C).

DISCUSSION

To analyze whether GLUT2 participates in the function of the hepatoportal glucose sensor, we evaluated the metabolic response to an intraportal glucose infusion in control and RIPGLUT1 \times GLUT2 $^{-/-}$ mice. Whereas a paradoxical hypoglycemic state developed in control mice, in RIPGLUT1 \times GLUT2 $^{-/-}$ mice, a transient hyperglycemic state was observed and was followed by a return to fasted glycemia. This glycemic pattern was similar to that obtained when glucose was infused through the femoral vein. At the same time, whole-body glucose turnover, and glycogen synthesis rates were no longer differentially affected by the portal and femoral glucose infusion protocols, and no inhibitory action of somatostatin on the portal vein glucose sensor could be observed. These data indicate that the hepatoportal sensor requires the presence of GLUT2, and we suggest that somatostatin action could involve these GLUT2-expressing cells.

To identify molecules that are involved in the hepatoportal glucose sensor, we first established an experimental protocol to permit activation of this sensor in the mouse. As a read-out, we used the development of hypoglycemia and the increase in whole-body glucose turnover in response to portal glucose infusion. To evaluate the role of GLUT2 in this glucose sensor, we used RIPGLUT1 \times GLUT2 $^{-/-}$ mice. In the

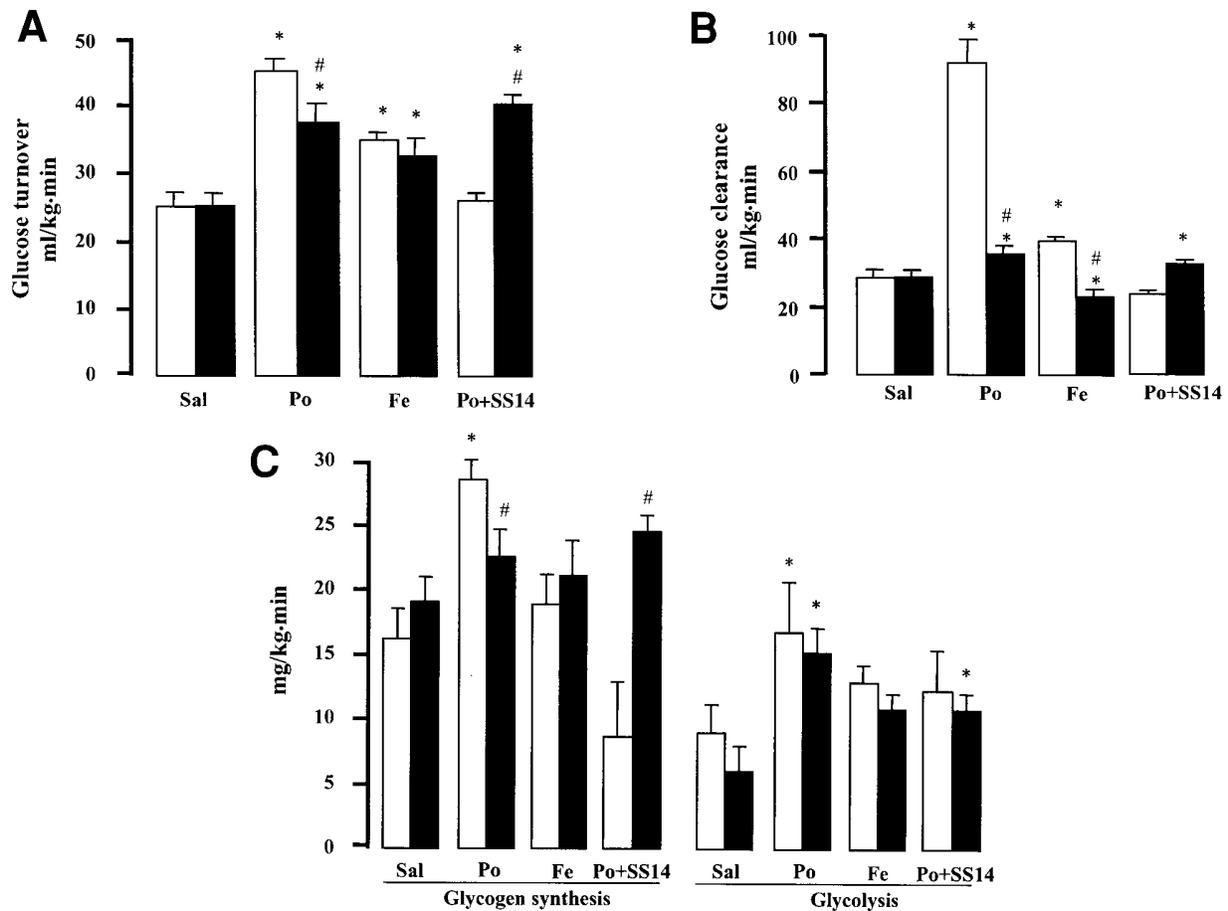


FIG. 2. Glucose turnover in control and RIPGLUT1×GLUT2^{-/-} mice. **A:** Glucose turnover was increased significantly more in Po- versus control Fe-mice. In RIPGLUT1×GLUT2^{-/-} mice, glucose turnover was increased to the same level in Po- and Fe-mice. Somatostatin infusion prevented the increase in glucose turnover in control Po-mice but not in RIPGLUT1×GLUT2^{-/-} Po-mice. No differences were observed between Sal-mice infused with and Sal-mice without somatostatin thus, only the Sal-mice data are presented. **B:** Calculation of glucose clearance further emphasizes the differences in glucose turnover between control and RIPGLUT1×GLUT2^{-/-} mice after activation of the hepatoportal glucose sensor. Somatostatin decreased glucose clearance in control Po- but not in mutant Po-mice. **C:** Whole-body glycogen synthesis in control mice was significantly increased compared with Sal-mice and control Po-mice but not in RIPGLUT1×GLUT2^{-/-} Po-mice. In addition, the increase in control mice was significantly greater than that in RIPGLUT1×GLUT2^{-/-} mice. The glycogen synthesis rate was not significantly different between the control and RIPGLUT1×GLUT2^{-/-} Fe-mice. Somatostatin decreased the whole-body glycogen synthesis rate in control Po-mice to the level of Sal-mice, but had no effect on the glycogen synthesis rate of the mutant Po-mice. Whole-body glycolysis was increased in both control and RIPGLUT1×GLUT2^{-/-} Po-mice compared with Sal-mice, whereas no significant increase was observed in the control and mutant Fe-mice. Somatostatin decreased the glycolysis rate in the control Po-mice and the RIPGLUT1×GLUT2^{-/-} mice. Data are means ± SE for 5–6 mice per group. **P* < 0.05 vs. Sal-mice; #*P* < 0.05 vs. control mice. □, Control mice; ■, RIPGLUT1×GLUT2^{-/-}.

absence of GLUT2, hypoglycemia did not develop in RIPGLUT1×GLUT2^{-/-} Po-mice. The lack of hypoglycemia development was not due to the absence of GLUT2 from hepatocytes and the consequent decrease in glucose uptake by this organ. Indeed, this lack of development was observed after transgenic reexpression of GLUT2 in the liver, an occurrence that did not restore sensitivity to the development of hypoglycemia during intraportal glucose infusion. This finding was not unexpected, because only a small fraction of infused glucose is normally cleared by the liver in the absence of hyperglycemia and hyperinsulinemia and, thus, does not cause a significant decrease in glucose concentration. Importantly, however, this result indicated that the participation of GLUT2 in the hepatoportal sensor was required to induce hypoglycemia. This conclusion was further sup-

ported by the observation that whole-body glucose turnover was markedly reduced in the absence of GLUT2. Therefore, the activation and signal transmission by the hepatoportal sensor depends on the presence of this transporter.

We reported in the preceding article that somatostatin co-infused in control Po-mice inhibited the stimulation of whole-body glucose turnover (11). In the absence of GLUT2, no effect of somatostatin could be observed on glucose utilization. The presence of somatostatin receptors in the portal vein has been reported by immunohistochemical analysis. These data revealed the presence of the receptor on nerve terminals located under the endothelial layer of the vein. These terminals may coexpress GLUT2. Alternatively, separate GLUT2-expressing cells may be located adjacent to the nerve terminals and may transmit a metabolic signal to the nerve

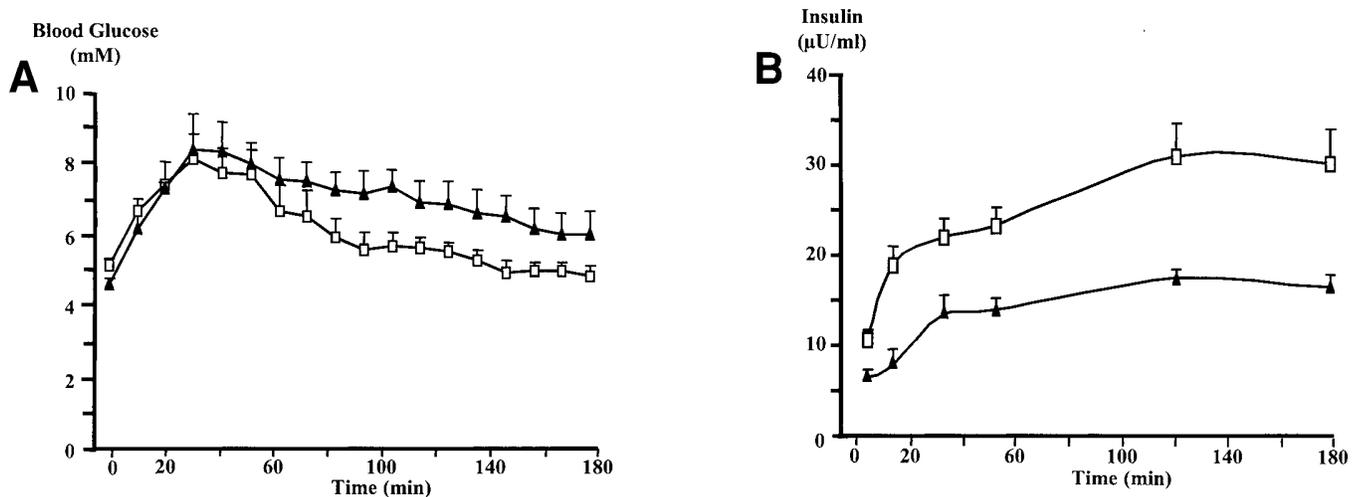


FIG. 3. Lack of somatostatin effect on glycemia and insulinemia in glucose-infused RIPGLUT1 × GLUT2^{-/-} mice. *A:* Somatostatin was co-infused with glucose in the portal vein of control or RIPGLUT1 × GLUT2^{-/-} mice. Somatostatin prevented hypoglycemia development in control mice but did not affect the glycemic pattern of RIPGLUT1 × GLUT2^{-/-} mice. Both curves were not statistically different by ANOVA. *B:* Insulinemic profiles of mice co-infused with glucose and somatostatin in the portal vein. Somatostatin did not prevent the increase in insulinemia observed in the absence of this hormone. The insulin profiles of RIPGLUT1 × GLUT2^{-/-} versus control mice in the presence of somatostatin were, however, statistically different by ANOVA with $P < 0.05$. Data are means \pm SE for 5–6 mice per group. □, Control mice; ▲, RIPGLUT1 × GLUT2^{-/-}.

endings. A similar metabolic coupling between neurons and glial cells has been proposed to be operative in the brain (17). The nature of the glucose-sensing cells and whether they are distinct from the nerve cells, which are electrically inactivated by increased glucose concentrations, is not known. They may, however, contain glucose-sensing molecules or surface receptors similar to those controlling or modulating glucose-induced insulin secretion by pancreatic β -cells. In support of this possibility and in addition to the presently proposed roles of GLUT2 and the somatostatin receptor, we observed that the insulinotropic hormone glucagon-like peptide 1 (GLP-1) can also activate the hepatic vagal afferents when injected directly into the portal vein (18). Also, in transgenic mice expressing the simian virus 40 large T antigen under the control of the glucokinase promoter, transformation of cells in the portal area was induced (19). Hence, there are cells in this location that normally express glucokinase, the key enzyme in the control of glucose-stimulated insulin secretion.

One unexpected finding was that glucose infusion in the femoral vein of RIPGLUT1 × GLUT2^{-/-} mice led to a glycemic profile that was significantly higher than that of Fe-control mice and was not accompanied by a parallel increase in insulinemia, even though islets from these mice showed restored first-phase insulin secretion in perfusion experiments and normal increases in insulinemia during hyperglycemic clamps (14). One possible explanation for this lack of an increase in insulin levels is that other glucose-sensing units, which are involved in the control of insulin secretion through hormonal or neural pathways, depend on the presence of GLUT2 (20). This transporter is indeed known to be present in the hypothalamus and the brainstem (21), where it may be associated with glucose-sensitive neurons that control β -cell-secretory activity; it may also be associated with other hormone-secreting cells, such as those that produce the glu-

coincretin glucose-dependent insulinotropic polypeptide and GLP-1. A decreased level of nervous input to the endocrine pancreas or decreased circulating levels of glucoincretins may, therefore, explain both hyperglycemia and hypoinsulinemia. The insulinotropic action of glucoincretins are more marked at glycemic levels slightly above normoglycemia than at high glucose concentrations (22). Thus, if GLUT2-dependent glucose-sensing units are involved in potentiating glucose-stimulated insulin secretion, their effect may be more marked at low glucose concentrations (5–8 mmol/l) than when glycemia is maintained at high levels (20 mmol/l) for prolonged periods of time (e.g., in clamp experiments). Thus, the difference in the glycemic pattern in control and mutant Fe-mice may be an indication that, in the normal situation, several glucose-sensing units participate in the control of insulin secretion and that they are GLUT2-dependent.

Further work is required to identify the cells that form the hepatoportal sensor and the molecules they express. However, the protocol presently developed for in situ metabolic evaluation in the mouse will allow us to assess the role of several genes, including those known to participate in glucose-induced insulin secretion by pancreatic β -cells and those from which gene knockout mice have been generated.

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