

Glucose Competence of the Hepatoportal Vein Sensor Requires the Presence of an Activated Glucagon-Like Peptide-1 Receptor

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Activation of the hepatoportal glucose sensors by portal glucose infusion leads to increased glucose clearance and induction of hypoglycemia. Here, we investigated whether glucagon-like peptide-1 (GLP-1) could modulate the activity of these sensors. Mice were therefore infused with saline (S-mice) or glucose (P-mice) through the portal vein at a rate of 25 mg/kg · min. In P-mice, glucose clearance increased to 67.5 ± 3.7 mg/kg · min as compared with 24.1 ± 1.5 mg/kg · min in S-mice, and glycemia decreased from 5.0 ± 0.1 to 3.3 ± 0.1 mmol/l at the end of the 3-h infusion period. Coinfusion of GLP-1 with glucose into the portal vein at a rate of 5 pmol/kg · min (P-GLP-1 mice) did not increase the glucose clearance rate (57.4 ± 5.0 ml/kg · min) and hypoglycemia (3.8 ± 0.1 mmol/l) observed in P-mice. In contrast, coinfusion of glucose and the GLP-1 receptor antagonist exendin-(9-39) into the portal vein at a rate of 0.5 pmol/kg · min (P-Ex mice) reduced glucose clearance to 36.1 ± 2.6 ml/kg · min and transiently increased glycemia to 9.2 ± 0.3 mmol/l at 60 min of infusion before it returned to the fasting level (5.6 ± 0.3 mmol/l) at 3 h. When glucose and exendin-(9-39) were infused through the portal and femoral veins, respectively, glucose clearance increased to 70.0 ± 4.6 ml/kg · min and glycemia decreased to 3.1 ± 0.1 mmol/l, indicating that exendin-(9-39) has an effect only when infused into the portal vein. Finally, portal vein infusion of glucose in GLP-1 receptor^{-/-} mice failed to increase the glucose clearance rate (26.7 ± 2.9 ml/kg · min). Glycemia increased to 8.5 ± 0.5 mmol/l at 60 min and remained elevated until the end of the glucose infusion (8.2 ± 0.4 mmol/l). Together, our data show that the GLP-1 receptor is part of the hepatoportal glucose sensor and that basal fasting levels of GLP-1 sufficiently activate the receptor to confer maximum glucose competence to the sensor. These data demonstrate an important extrapan-

creatic effect of GLP-1 in the control of glucose homeostasis. *Diabetes* 50:1720–1728, 2001

After a meal, glucose is absorbed by the intestine and collected into the hepatoportal vein. A positive glucose concentration gradient between the hepatoportal vein and arterial blood is thus established. Glucose-sensitive units present in the hepatoportal vein detect this gradient (1–3) and send a signal through the hepatic branch of the vagus nerve to target tissues such as the liver (4–8), the hypothalamus (9,10), insulin-secreting β -cells (11), the brain stem (12), and the adrenal glands (13,14). The specific cellular functions that are activated then participate in the adaptation of the body to the new metabolic situation. We previously described that one of the consequences of activating the hepatoportal glucose sensor was an increase in blood glucose clearance and utilization in a subset of tissues, mostly heart, soleus, and brown adipose tissue (15). We also showed that activation of this sensor was inhibited by somatostatin and that it required the presence of the glucose transporter GLUT2 (16).

The best-described glucose sensing system is the insulin-secreting pancreatic β -cells (17). In these cells, glucose induces insulin secretion by a mechanism that depends on glucose metabolism. This secretory activity can be strongly potentiated by hormones such as the glucocorticoid glucagon-like peptide-1 (GLP-1). This hormone is secreted postprandially by intestinal L-cells in the portal vein and reaches the pancreatic β -cells, where it binds to a specific plasma membrane G protein-coupled receptor linked to the adenylyl cyclase pathway. The consequent rise in intracellular cAMP content potentiates the glucose signal, leading to insulin secretion (18–20). Based on the β -cell model, we hypothesized that GLP-1 could also modulate the activity of the hepatoportal glucose sensor. This hypothesis was supported by the finding that GLP-1 can control the firing rate of the hepatic branch of the vagus nerve, which is controlled by the hepatoportal glucose sensor (21,22). The active form of GLP-1 is GLP-1-(7-37) or GLP-1-(7-36)amide; it is also intriguing that the half-life of GLP-1-(7-37) and GLP-1-(7-36)amide is very short (~0.5 min) because of a rapid proteolytic cleavage of the first two NH₂-terminal amino acids by dipeptidylpep-

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AUC, area under the curve; Ex, infused with exendin-(9-39); F, infused with glucose in the femoral vein; -GLP-1, coinfused with glucagon-like peptide-1 and glucose in the femoral vein; GLP-1, glucagon-like peptide-1; GLP-1R^{-/-}, GLP-1-receptor^{-/-}; P, infused with glucose in the portal vein; P-fEx, infused with exendin-(9-39) through the femoral vein and glucose through the portal vein; P-GLP-1, coinfused with GLP-1 and glucose in the portal vein; S, infused with saline; S-GLP-1, coinfused with GLP-1 and saline.

tidase IV. Capillaries adjacent to GLP-1-secreting L-cells have been shown to produce a high level of this enzyme (23), suggesting an explanation for the very rapid inactivation of this peptide. This observation also suggests that the hepatoportal sensor may be the target exposed to the highest circulating concentration of active GLP-1. Recently, it has been shown that coinfusion of GLP-1 and glucose in the portal vein increases insulin secretion through activation of a neuronal mechanism (24).

Here, we analyze the role of GLP-1 and its receptor in the regulation of the hepatoportal vein glucose sensor. The induction of hypoglycemia and the increase in glucose clearance induced by portal-vein glucose infusion were not modified by coinfusion of GLP-1, although they were suppressed by the GLP-1 receptor agonist exendin-(9-39). This impaired induction of hypoglycemia and activation of glucose clearance were also observed when glucose was infused in the portal vein of GLP-1 receptor^{-/-} mice (GLP-1R^{-/-} mice). Therefore, these data suggest that occupation of the GLP-1 receptor by its ligand is required to maintain the glucose competence of the hepatoportal glucose sensor, thereby identifying a novel extrapancreatic function of GLP-1 in the control of glucose homeostasis.

RESEARCH DESIGN AND METHODS

Surgical procedure for catheter implantation. Under pentobarbital anesthesia (65 mg/kg i.p.), a first catheter was implanted into the hepatoportal vein through the splenic vein of 12- to 16-week-old C57BL6 mice (IFFA-CREDO, L'arbresle, France) as well as GLP-1 receptor knockout mice, as previously described (25). After 4–6 days, allowing for full body-weight recovery, one extremity of a second catheter was implanted into the femoral vein under similar anesthetic conditions. The other extremity of the catheter was tunneled under the skin of the back, exteriorized, and secured in place at the back of the neck. The mice were then allowed to recover from the surgery in individual cages. The GLP-1R^{-/-} mice were of a mixed CD1 and SV129 background and have been extensively characterized (25). Control mice were C57BL6, a strain that behaved in a manner similar to C57BL/6xSV129 in our present glucose infusion protocol (15).

Infusion procedures. At 5–7 days after catheter implantations, the mice were fasted for 6 h. A 33% glucose solution was infused for 180 min through either the portal or femoral vein at a rate of 2 μ l/min (corresponding to \sim 25 mg/kg \cdot min), which corresponds to the mean value of the endogenous production rate in 6-h fasted 30-g mice (15). A saline solution was infused through either the portal or femoral vein (S-mice) at a rate of 2 μ l/min. Either GLP-1, in the form of GLP-1-(7-36)amide (Bachem, Bubendorf, Switzerland), or exendin-(9-39) (a gift from Dr. John Eng) was infused into the portal vein at rates ranging from, respectively, 0.5 to 50 pmol/kg \cdot min (P-GLP-1 mice) or 0.05 to 50 pmol/kg \cdot min (P-Ex mice).

To determine the whole-body glucose clearance rate, high-performance liquid chromatography-purified D-(³H)3-glucose (NEN LifeScience, Boston, MA) was infused continuously through the femoral vein at a rate of 10 μ Ci/kg \cdot min. Plasma glucose and D-(³H)3-glucose concentrations were determined in 5 μ l of blood sampled from the tip of the tail vein 20 min after the initiation of infusion (15). A steady specific activity with variations <15% was obtained during the last hour of the infusion. Mice showing larger variations in the specific activity were excluded from the study.

Analytical procedures. Blood glucose concentration was determined during the infusions using a glucose meter (Roche Diagnostic, Rotkreuz, Switzerland). Plasma insulin was determined using an enzyme-linked immunosorbent assay kit (Crystal Chem, Chicago, IL) before and at 10, 30, 50, 120, 180 min after the beginning of the infusions in 5 μ l of plasma from 15 μ l of blood collected from the tail tip (15). Intra- and interassay coefficients of variation were <5 and <15%, respectively.

Isotope measurements. Plasma D-(³H)3-glucose enrichment was determined from total blood after deproteinization (15), which was performed using the following procedure. First, 5 μ l of tail venous blood was mixed with a 0.3-mol/l ZnSO₄ solution. Then, a 0.3-mol/l Ba(OH)₂ solution was added to precipitate the proteins and blood cells, and the Zn(OH)₂ precipitate formed was spun down. An aliquot of the supernatant was evaporated to dryness to determine the radioactivity corresponding to D-(³H)3-glucose. In another aliquot of the same supernatant, the glucose concentration was assessed using the glucose

oxidase method (Trinder; Sigma, St. Louis, MO). The recovery of the assay was monitored for each experiment. To determine the D-(³H)3-glucose infusion rate, an aliquot of the D-(³H)3-glucose infusate was treated under the same conditions as above. A 90–95% recovery of the D-(³H)3-glucose was usually obtained. In addition, an aliquot of an internal control was processed for each type of analysis. This standard corresponds to an aliquot from a plasma pool that contains a defined amount of D-(³H)3-glucose.

Calculations. Calculations were made with parameters collected during the last 60 min of the infusions, when a steady state D-(³H)3-glucose enrichment was obtained. First, glucose turnover was calculated by dividing the D-(³H)3-glucose infusion rate by the plasma glucose specific activity, as previously described (15). The mean was calculated for each time point of this period. Second, whole-body glucose clearance (in milliliters per kilogram \times minute) was calculated by dividing the mean glucose turnover rate by the mean blood glucose concentration obtained during the last hour of the infusion. The mean value of mice from the same group was reported. The area under the curve (AUC) was calculated for blood glucose and plasma insulin concentrations obtained during the infusion period. To calculate the blood glucose AUC of each mouse, the fasting value was subtracted from each concentration of glucose determined 20 min after the beginning of the infusion and every 20 min until completion. The new values were multiplied by 20 min, which corresponds to the time period between two samplings. To calculate the area under the plasma insulin curve, the fasted insulinemia was subtracted from each of the values determined at 10, 30, 50, 120, and 180 min, and these values were multiplied by the time length (in minutes) between two samplings. These data were then added together to obtain the AUC.

Statistical analysis. Results are presented as the means \pm SE. Statistical significance of differences were analyzed by using Student's *t* test for unpaired bilaterally distributed values of equal variance. Values were considered different from each other at *P* < 0.05.

RESULTS

Activation of the hepatoportal sensor stimulates glucose clearance. Portal glucose infusion at a rate equivalent to endogenous glucose production progressively induced a decrease in blood glucose levels that reached 3.3 ± 0.1 mmol/l after 180 min, as shown in Fig. 1A. In contrast, when glucose was infused at the same rate through the femoral vein (F-mice), a transient hyperglycemia was observed. Infusion of saline through the portal vein did not modify the basal glycemic levels. The AUC relative to the basal glycemic levels indicates a significant decrease in the glycemic profile in P-mice (-138 ± 28 mmol/l \cdot min) and a significant increase in F-mice (324 ± 50 mmol/l \cdot min) compared with S-mice (22 ± 58 mmol/l \cdot min) (Fig. 1A, inset). Blood glucose clearance (Fig. 1B) was strongly increased in P-mice (67.5 ± 3 ml/kg \cdot min) compared with both S- and F-mice (27.1 ± 1.5 ml/kg \cdot min and 30.7 ± 1.7 ml/kg \cdot min, respectively). The difference in glycemic profiles and glucose clearance between P- and F-mice could not be explained by differences in insulin profiles or in insulin AUCs during the course of the experiments, as shown in Fig. 1C. These data indicate that activation of the hepatoportal sensor increased glucose clearance and induced hypoglycemia. They are similar to those previously reported (15).

GLP-1 infusion into the portal vein does not further increase stimulation of the hepatoportal sensor by glucose. To investigate whether GLP-1 could further activate the hepatoportal glucose sensor, we coinjected the peptide with glucose into the portal vein of control mice (P-GLP-1 mice) at a rate of 5 pmol/kg \cdot min. This increased plasma GLP-1 concentration to 69 ± 14 pmol/l, as assessed at completion of the infusion experiment. In these mice, hypoglycemia reached 3.8 ± 0.1 mmol/l at 180 min of infusion. Coinfusion of GLP-1 in portal saline-infused or in femoral glucose-infused mice led to glycemic profiles and AUCs that were not different from those obtained when GLP-1

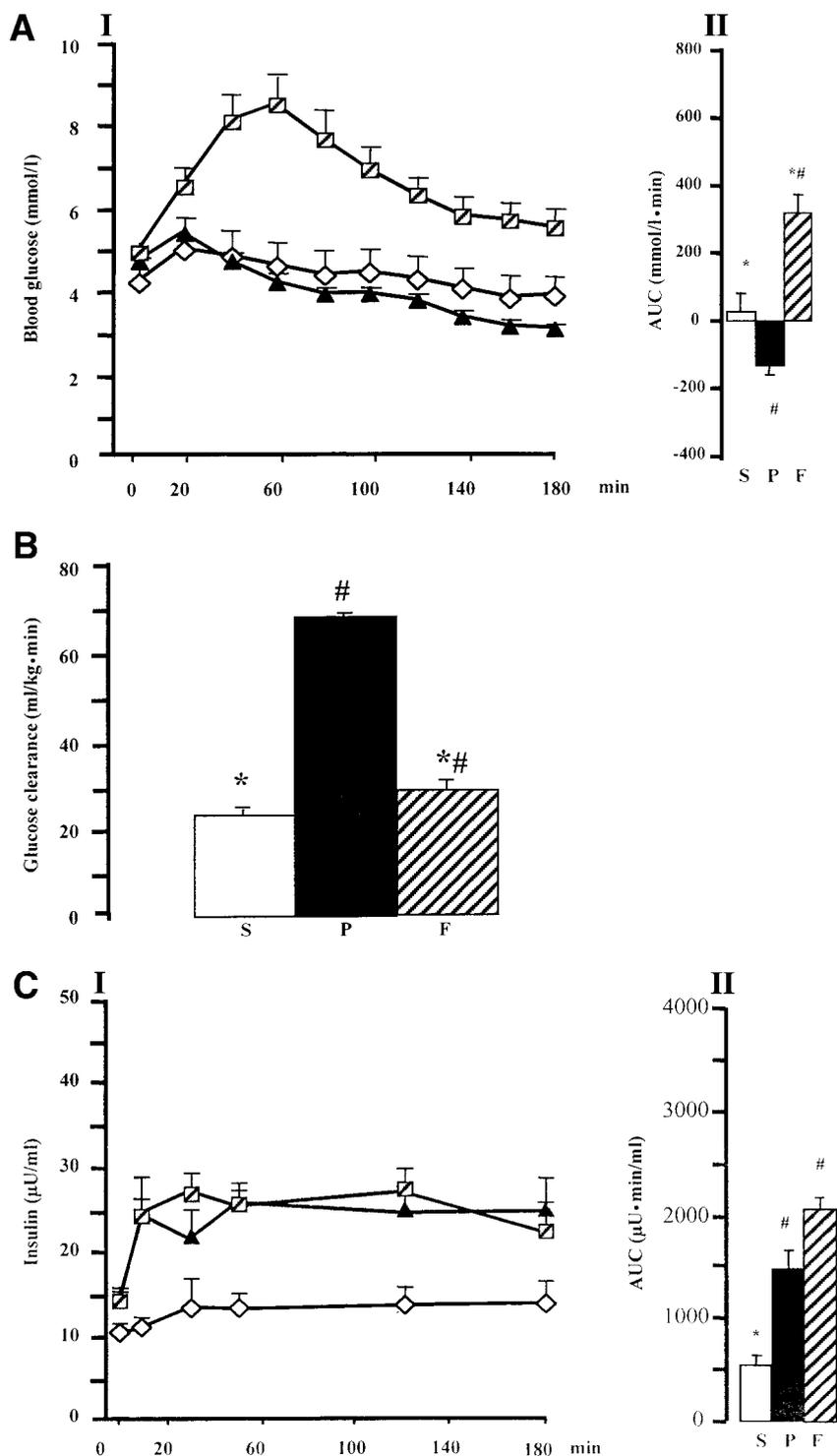


FIG. 1. Activation of the hepatoportal glucose sensor induces hypoglycemia and increased blood glucose clearance. Glucose was infused through the portal (P, ▲) or the femoral (F, ▨) vein of mice at a rate of 25 mg/kg · min. Data were compared with saline-infused mice (S, ◇). Because no differences were noted between mice infused with saline into the portal or the femoral vein, the data were pooled. **A:** Glycemic pattern of the infused mice (I). The mean AUC of the blood glucose concentration relative to the fasting values is also shown (II). Each data point is the means ± SE for $n \geq 12$ for each group. **B:** Blood glucose clearance calculated during the last hour of the infusion. **C:** Insulinemia during the glucose or saline infusion protocols (I). The mean AUC of the plasma insulin concentrations relative to the fasting values is also shown (II). Data are the means ± SE for five to six mice per group. *Statistically different from P-mice; #statistically different from S-mice; $P < 0.05$.

was omitted (Fig. 2A and compare with Fig. 1A). The blood glucose AUCs relative to the basal values are shown in Fig. 2A (inset). Blood glucose clearance was increased to 57.4 ± 5.0 ml/kg · min in P-GLP-1 mice, which is significantly higher than in S-GLP-1 and F-GLP-1 mice ($24.9 \pm$

2.6 and 32.0 ± 1.3 ml/kg · min, respectively) (Fig. 2B) but not different from that of P-mice (67.5 ± 3 ml/kg · min) (Fig. 1B). However, the insulinemic profiles and insulin AUCs were higher when GLP-1 was coinfused into the portal vein as compared with the femoral vein (AUC $3,570 \pm$

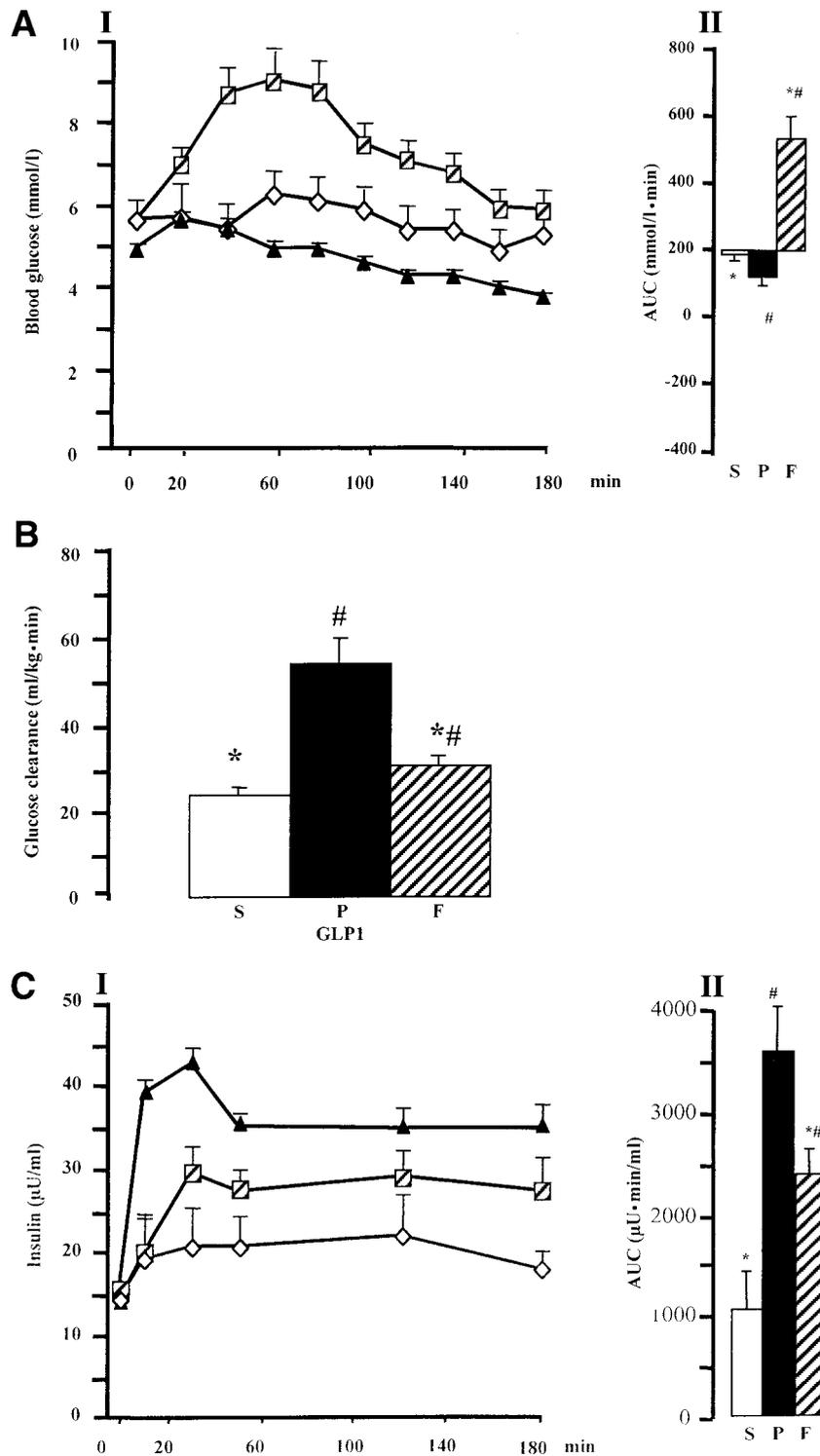


FIG. 2. No effect of exogenous GLP-1 on activation of the hepatoportal glucose sensor. Glucose was infused at a rate of 25 mg/kg · min and GLP-1 was infused at a rate of 5 pmol/kg · min through the portal (P-GLP-1, ▲) or the femoral (F-GLP-1, ▨) vein of mice. Data were compared with saline infused mice (S-GLP-1, ◇). **A:** Glycemic pattern of the infused mice (I). The mean AUC of the blood glucose concentration values relative to the fasting values is also shown (II). Each data point is the mean \pm SE for $n \geq 6$ for each group. **B:** Blood glucose clearance calculated during the last hour of the infusion. **C:** Insulinemia during the glucose or saline infusion protocols (I). The mean AUC of the blood glucose concentration values relative to the fasting values is also shown (II). Data are the means \pm SE for five to six mice per group. *Statistically different from P-GLP-1 mice; #statistically different from S-GLP-1 mice; $P < 0.05$.

472 vs. $2,352 \pm 263$ $\mu\text{U} \cdot \text{min}/\text{ml}$, respectively) (Figs. 2C and 1C, insets). When GLP-1 was coinfused with glucose into the portal vein at rates of 0.5 or 50 pmol/kg · min ($n = 5$, data not shown), the same glycemic profiles and glucose clearance rates were obtained as when the infusion rate

was 5 pmol/kg · min. These data indicate that exogenous infusion of GLP-1 in the portal vein did not modify the activity of the hepatoportal glucose sensor, as measured by the induction of hypoglycemia and the increase in glucose clearance, but it increased insulin AUCs.

Portal infusion of exendin-(9-39) prevents activation of the hepatoportal glucose sensor. Because GLP-1 is released by intestinal L-cells directly into the portal vein, we hypothesized that the absence of effect of exogenous GLP-1 on the hepatoportal sensor could be because of an occupancy of the receptor producing a maximal effect on the sensor. Exendin-(9-39), which is an inverse agonist of the GLP-1 receptor (26,27), was therefore coinjected with glucose into the portal vein (P-Ex mice). Coinjection of exendin-(9-39) into the portal vein at a rate of 0.05 pmol/kg · min did not consistently inhibit the increased glucose clearance rate (not shown). When this rate of coinjection was increased to 0.5 pmol/kg · min, there was a complete suppression of the hypoglycemia-inducing effect, and instead, glycemia rose from 5.7 ± 1 to 9.2 ± 0.3 mmol/l 60 min after the beginning of the infusion and progressively returned to the fasting value of 5.6 ± 0.3 mmol/l at the end of the infusion period (Fig. 3A). The blood glucose AUC of the P-Ex mice was 244 ± 24 mmol/l · min, significantly higher than the corresponding value for P-mice (-138 ± 28 mmol/l · min) (Fig. 3A inset, compare with Fig. 1B inset). When exendin-(9-39) was coinjected with glucose into the portal vein at rates of 5 or 50 pmol/kg · min ($n = 8$ and 4, respectively, data not shown), the same glycemic profiles and glucose clearance rates were obtained as when the infusion rate was 0.5 pmol/kg · min. Importantly, however, we found that when glucose was infused into the portal vein and exendin-(9-39) was infused into the femoral vein at the rate of 0.5 pmol/kg · min, increases in glucose clearance and hypoglycemia were still induced. Therefore, this strongly suggests that the primary site of action of exendin is on the hepatoportal sensor itself. Infusion of exendin-(9-39) with saline into the portal vein only minimally affected the glycemic profile, but infusion into the femoral vein with glucose prevented the return of glycemia to the basal value after it had reached a maximal level of 9.4 ± 0.9 mmol/l 60 min after the beginning of the infusion (Fig. 3A).

The effect of exendin-(9-39) on the hepatoportal sensor was further evidenced when measuring glucose clearance rates and insulin profiles. Glucose clearance was markedly reduced in P-Ex mice (36.1 ± 2.6 ml/kg · min) compared with P- and P-GLP-1 mice (67.5 ± 3 and 57.4 ± 5.0 ml/kg · min, respectively), although it was still significantly higher than when the mice were infused with exendin-(9-39) alone (S-Ex mice, 27.7 ± 2.1 ml/kg · min). Importantly, the glucose clearance of mice receiving exendin-(9-39) through the femoral vein and glucose through the portal vein (P-fEx mice) was still increased to a level similar to that seen in P-mice and P-GLP-1 mice (Fig. 3B, compare with Figs. 1B and 2B). Finally, the insulin profiles of the P-Ex mice and F-Ex mice were similar, as were their insulin AUCs (Fig. 3C). However, a significant reduction in insulin AUC was obtained in P-fEx mice compared with P-Ex mice, even though glucose clearance values were similar.

Lack of stimulation by glucose of the hepatoportal glucose sensor in GLP-1 receptor^{-/-} mice. To further evaluate the role of GLP-1 and its receptor in the function of the hepatoportal glucose sensor, we performed glucose infusion and metabolic measurements in GLP-1-receptor^{-/-} mice. When glucose was infused into the portal vein

of mutant mice (P-GLP-1R^{-/-} mice), the blood glucose concentration increased from 6.0 ± 0.5 to 8.5 ± 0.5 mmol/l 60 min after the beginning of the infusion and remained elevated until the end of the infusion period (Fig. 4A). This profile is similar to that of P-Ex mice, except that in these mice, glycemia progressively returned to basal values (Fig. 3A). The profile of P-GLP-1R^{-/-} mice was, however, similar to that of mutant mice infused with glucose and exendin-(9-39) through the femoral vein. The blood glucose clearance in P-GLP-1R^{-/-} mice was not increased over the value of the S-GLP-1R^{-/-} mice (26.7 ± 2.9 and 28.7 ± 0.5 ml/kg · min, respectively) and was also similar to that of F-GLP-1R^{-/-} mice (Fig. 4B). Fasting plasma insulin levels were higher in mutant than in control mice. Insulinemic levels were nevertheless still increased by either portal or femoral glucose infusion (Fig. 4C).

DISCUSSION

In the present study, we evaluated the role of GLP-1 and its receptor on the activation of the hepatoportal glucose sensor. We show that portal coinjection of GLP-1 with glucose did not further activate the hepatoportal sensor. In contrast, coinjection into the portal vein of the receptor antagonist exendin-(9-39) with glucose strongly inhibited its activation. Finally, studies with GLP-1 receptor-null mice also showed loss of glucose sensor activation. These data demonstrate that the GLP-1 receptor is required for the function of the hepatoportal glucose sensor and that, in the fasted state, it is already sufficiently activated to maintain the glucose competence of the sensor.

We recently reported that stimulation of the hepatoportal glucose sensor by a portal glucose infusion induced hypoglycemia associated with an increase in glucose clearance. This resulted, at least in part, from stimulation of glucose utilization in a subset of tissues including soleus muscle, heart, and brown adipose tissue (15). We further demonstrated that activation of this sensor could be inhibited by somatostatin and that it was dependent on the presence of GLUT2 (16). These data revealed an important function of the hepatoportal sensor in the control of glucose homeostasis, which may be particularly active in the postprandial state, when absorbed nutrients rapidly appear in the portal circulation. These data also suggested a similarity between glucose sensing by this portal sensor and the pancreatic β -cells. To further study this possible similarity, we evaluated whether GLP-1, the most potent insulinotropic hormone known so far, could also participate in the activation of the portal sensor. Our first experiments were therefore aimed at evaluating whether coinjection of GLP-1 and glucose into the portal vein would further increase the activation of the portal sensor by glucose. If this were the case, hypoglycemia and the glucose clearance rate would be increased. In contrast to these expected results, portal coinjection of GLP-1 with glucose did not change any of these parameters. This was observed with a GLP-1 infusion rate of 5 pmol/kg · min (which increases plasma GLP-1 levels to threefold the fasting values) as well as with a higher infusion rate of 50 pmol/kg · min.

These results could be interpreted in two ways. First, it is possible that GLP-1 and its receptor are not involved in the function of the glucose sensor. Second, fasting GLP-1

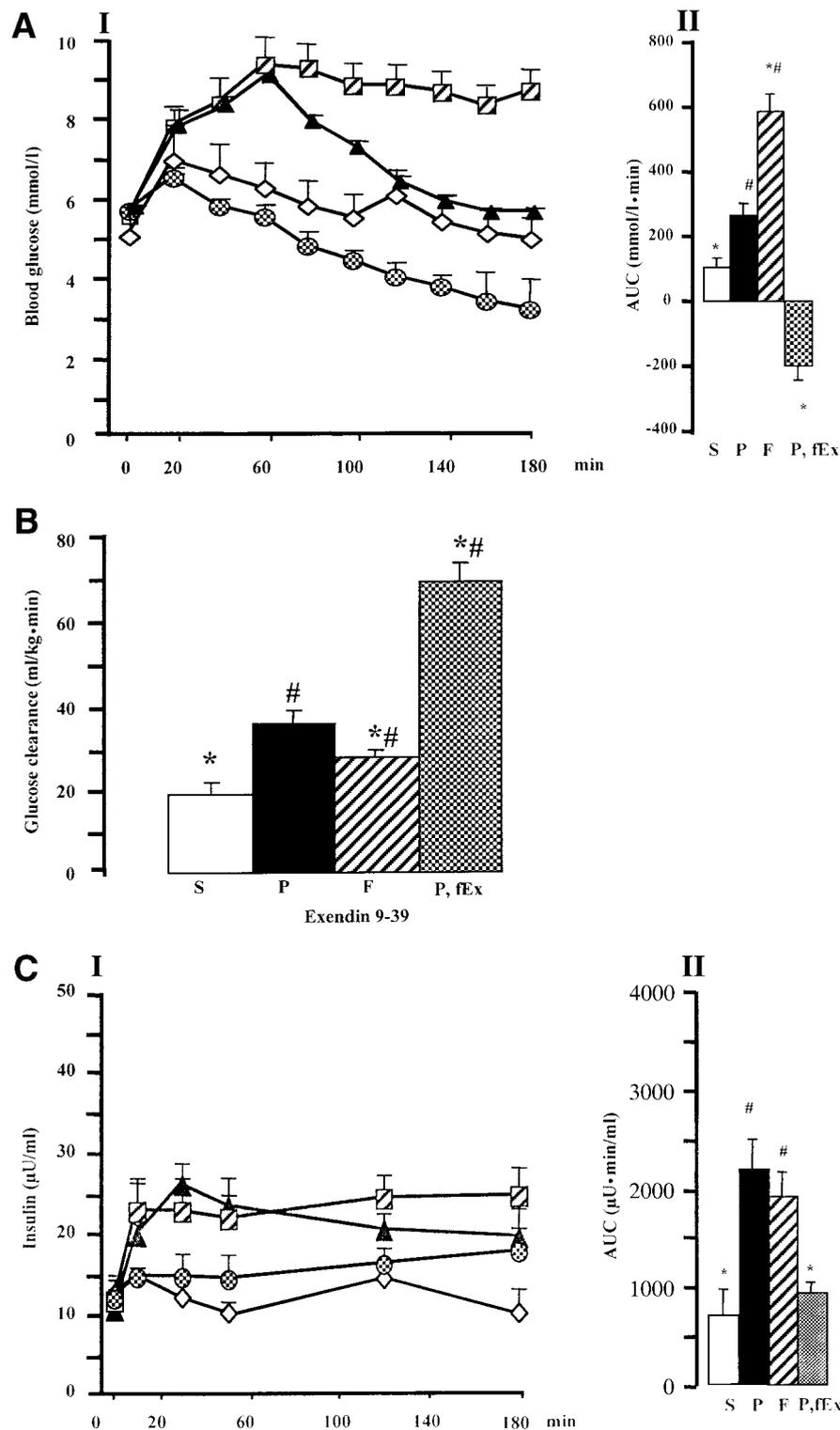


FIG. 3. Exendin-(9-39) prevents activation of the hepatoportal glucose sensor. Glucose was infused at a rate of 25 mg/kg · min and exendin-(9-39) was infused at a rate of 0.5 pmol/kg · min through the portal (P-Ex, ▲) or the femoral (F-Ex, ▨) vein of mice, or glucose was infused into the portal vein and exendin-(9-39) was infused into the femoral vein (P-fEx, ●). Data were compared with S-Ex mice (◇). **A:** Glycemic pattern of the infused mice (**I**). The mean AUC of the blood glucose concentration values relative to the fasting values is also shown (**II**). Each data point is the mean \pm SE for $n \geq 6$ for each group. **B:** Blood glucose clearance rate calculated during the last hour of the infusion. **C:** Insulinemia during the glucose or saline infusion protocols (**I**). The mean AUC of the blood glucose concentration values relative to the fasting values is also shown (**II**). Data are the means \pm SE for five to six mice per group. *Statistically different from P-Ex mice; #statistically different from S-Ex mice; $P < 0.05$.

levels may be sufficient to activate the receptor to confer maximal sensitivity of the hepatoportal sensor to glucose. To discriminate between these two possibilities, exendin-(9-39) was coinjected with glucose into the hepatoportal

vein. Strikingly, this led to a strong inhibition of the portal sensor, as revealed by the suppression of both the hypoglycemia-inducing effect and the increased glucose clearance. This effect was already at maximum, with a rate of

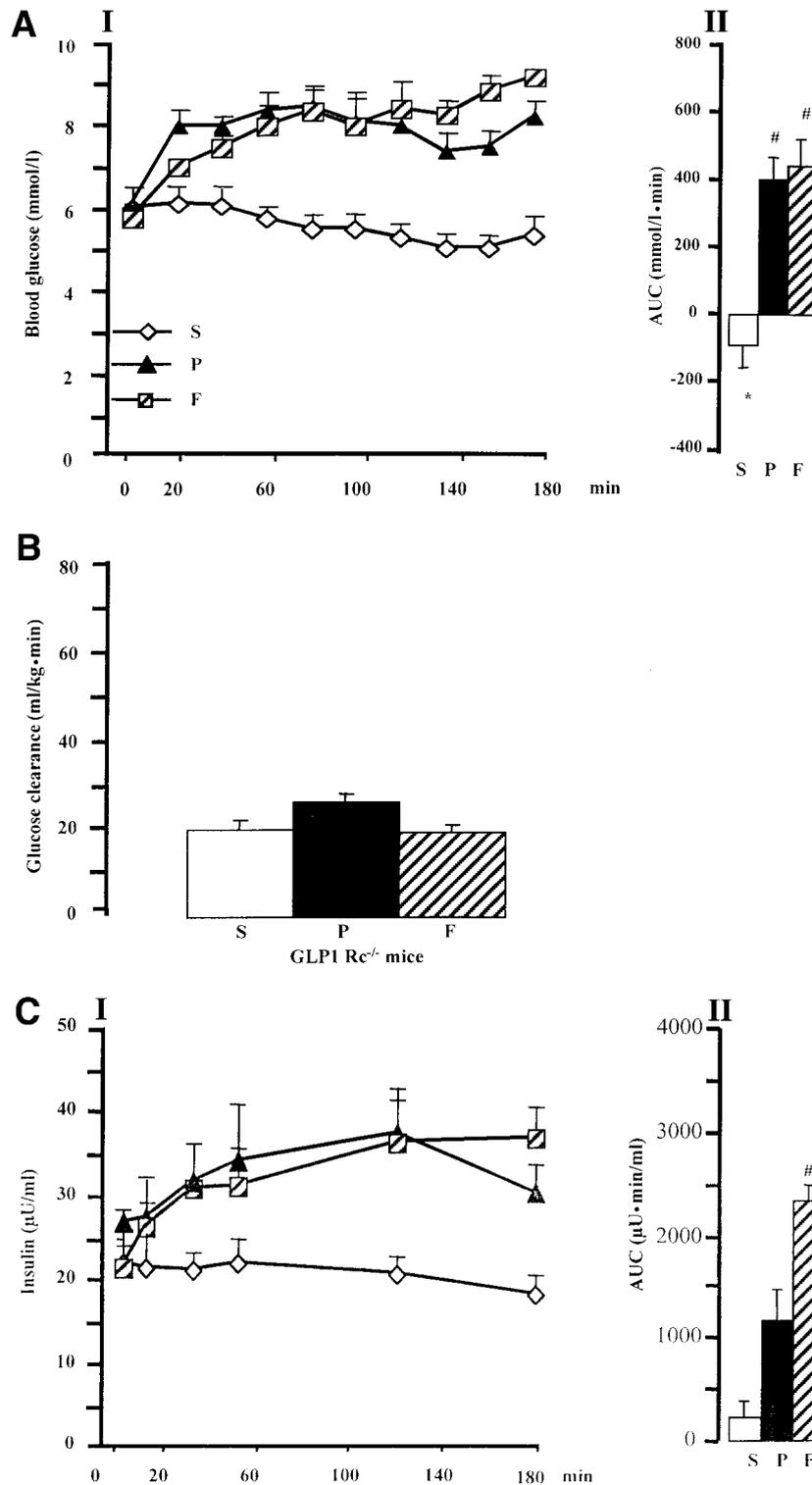


FIG. 4. No activation of the hepatoportal glucose sensor in GLP-1-Rc^{-/-} mice. Glucose was infused through the portal (P, ▲) or the femoral (F, ◻) vein of mice at a rate of 25 mg/kg · min. Data were compared with saline-infused mice (S, ◇). **A:** Glycemic pattern of the infused mice (I). **B:** Blood glucose clearance calculated during the last hour of the infusion. **C:** Insulinemia during the glucose or saline infusion protocols (I). The mean AUC of the blood glucose concentration values relative to the fasting values are also shown (A-II and C-II). Data are the means ± SE for five to six mice per group. *Statistically different from P-mice; #statistically different from S-mice; P < 0.05.

infusion of 0.5 pmol/kg · min. It could not be explained simply by a decrease in insulin secretion because the insulin AUC over the period of infusion was similar whether exendin-(9-39) was present or absent. To evaluate whether exendin-(9-39) was acting on the portal sensor

and not on GLP-1 receptors present on other glucose sensors, we infused glucose through the portal vein and exendin-(9-39) through the femoral vein at the rate of 0.5 pmol/kg · min. We expected that under these conditions, if the peptide was acting on the portal sensor, its infusion

through the femoral vein would lead to a sufficient dilution of its circulating concentration such that no inhibition of the portal sensor could be obtained. This is indeed what we observed; hypoglycemia and increased glucose clearance rate were not inhibited. Therefore, we conclude that there is a direct effect of exendin-(9-39) on inhibition of the portal vein glucose sensor.

Interestingly, exendin-(9-39), when coinjected with glucose into the portal or the femoral vein, did not reduce plasma insulin concentration, as could have been expected from the antagonist activity of this peptide. However, under these conditions, hyperglycemia developed and may have a dominant effect on the stimulation of insulin secretion.

What is the mechanism by which exendin-(9-39) inhibits the portal glucose sensor? GLP-1 is secreted by L-cells located primarily in the distal small intestine and the colon and is collected into the portal circulation before being distributed to the periphery, including to pancreatic β -cells. Therefore, one can suppose that the portal sensor is exposed to the highest plasma concentration of GLP-1. This may be even more true when we consider that GLP-1 is rapidly inactivated in the plasma by dipeptidylpeptidase IV, an enzyme that is found at particularly high levels in those endothelial cells forming capillaries found in close proximity to L-cells (23). This therefore suggests that degradation of the active peptide may be very rapidly initiated after its secretion but that GLP-1 may be present at a higher concentration in the portal vein than in peripheral blood. Thus, even in the fasted state, there may be a sufficient concentration of intact GLP-1 that reaches the portal sensor to maintain it in a maximally glucose-competent state. Exendin-(9-39) would then act by displacing the peptide from its receptor. An alternative explanation may be derived from our previous studies, which demonstrated that in β -cells, the GLP-1 receptor had a basal ligand-free activity and that exendin-(9-39) acted as an inverse agonist, decreasing basal cAMP levels and glucose-stimulated insulin secretion (27). We can therefore propose that exendin-(9-39) may also act as an inverse agonist, reducing the activity of the ligand-free receptor present in the glucose sensor.

Interestingly, the P-GLP-1 and P-mice had similar glucose clearance rates, but the former group had a higher plasma insulin level, suggesting that either the small rise in insulin had no consequence on the glucose clearance or that insulin resistance was activated by portal GLP-1 infusion. Similarly, previous data (24) showed that an intraportal injection of a small dose of GLP-1 could increase insulin secretion, an effect not observed when the same dose of GLP-1 was injected through the jugular vein. However, despite these different insulinemic levels, the glycemic patterns were the same with or without GLP-1 injection, also suggesting that insulin-induced glucose clearance was reduced by GLP-1 injection.

The observation that the GLP-1 receptor was involved in the control of the hepatoportal glucose sensor was further investigated in GLP-1R^{-/-} mice. We showed that portal glucose infusion failed to activate glucose clearance and that it also failed to induce hypoglycemia. Because these mice have a general inactivation of the receptor, which also potentiates insulin secretion, it was critical to evalu-

ate the insulin profiles during the time course of the experiment. Our data show that basal insulinemia of the fasted mutant mice was higher than that of control mice. In addition, portal or femoral glucose infusion increased plasma insulin to similar levels above those of the saline-infused mutant mice. However, activation of glucose clearance and induction of hypoglycemia were no longer detectable after stimulation of the glucose sensor by glucose. We therefore take these observations as a further indication that the activation of the hepatoportal sensor requires expression of the GLP-1 receptor.

Our observations indicate that exogenous GLP-1 infusion in the portal vein does not increase glucose clearance. This could suggest that the hepatoportal sensor is not normally modulated by postprandial elevations in GLP-1, which would be in contrast to other reports showing that portal GLP-1 infusion stimulates the sensor. Indeed, activation of the sensor by a portal-peripheral glucose gradient decreases the firing rate of vagal afferents originating from the portal vein (1), and portal infusion of GLP-1 increased this firing rate (22,28). Although these data suggest a modulation of the sensor by GLP-1, the induced change in firing activity is opposite of that change triggered by glucose, indicating that GLP-1 should oppose the glucose effect rather than stimulate it. In contrast, here we find that the effect of glucose was inhibited by the GLP-1 antagonist exendin-(9-39). Furthermore, we previously showed that somatostatin blocked the effect of the activated portal sensor on stimulation of glucose utilization (15), whereas Nakabayashi et al. (22,28) reported that somatostatin increased the firing rate of the afferent branches of the vagal nerve, similar to GLP-1. Thus, whereas these two hormones have opposite effects on glucose clearance, they have the same effect on firing rates. Therefore, the effect of the activated portal sensor may not be transmitted uniquely by the vagal afferences. In this context, our previous data (15) showed that hindlimb denervation only partly prevented the effect of the activated hepatoportal glucose sensor on stimulation of glucose utilization, suggesting that the link between the sensor and target tissues may involve multiple neural mechanisms. Alternatively, the portal glucose sensor may be connected to multiple efferent pathways that control different physiological functions, and these pathways may be activated by distinct GLP-1 concentrations.

Finally, because the hepatoportal glucose sensor shows similarities with the pancreatic β -cells, it can be hypothesized that a defect in glucose sensing by this sensor may also develop in diabetes and accentuate the diabetic syndrome. Because the sensor is activated by a portal-peripheral glucose gradient, diabetic glycemia may prevent the establishment of this gradient and therefore prevent glucose sensing. Understanding the molecular mechanisms of hepatoportal glucose sensing, signal transduction, and activation of glucose uptake by peripheral tissues may lead to identification of novel therapeutic targets for the treatment of diabetes.

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