Evidence That Extrapancreatic GLUT2-Dependent Glucose Sensors Control Glucagon Secretion

Rémy Burcelin and Bernard Thorens

GLUT2$^{-/-}$ mice reexpressing GLUT1 or GLUT2 in their β-cells (RIPGLUT1 × GLUT2$^{-/-}$ or RIPGLUT2 × GLUT2$^{-/-}$ mice) have nearly normal glucose-stimulated insulin secretion but show high glucagonemia in the fed state. Because this suggested impaired control of glucagon secretion, we set out to directly evaluate the control of glucagonemia by variations in blood glucose concentrations. Using fasted RIPGLUT1 × GLUT2$^{-/-}$ mice, we showed that glucagonemia was no longer increased by hypoglycemic (2.5 mmol/l glucose) clamps or suppressed by hyperglycemic (10 and 20 mmol/l glucose) clamps. However, an increase in plasma glucagon levels was detected when glycemia was decreased to ≤1 mmol/l, indicating preserved glucagon secretory ability, but of reduced sensitivity to glucopenia. To evaluate whether the high-fed glucagonemia could be due to an abnormally increased tone of the autonomic nervous system, fed mutant mice were injected with the ganglionic blockers hexamethonium and chlorisondamine. Both drugs lead to a rapid return of glucagonemia to the levels found in control fed mice. We conclude that *1*) in the absence of GLUT2, there is an impaired control of glucagon secretion by low or high glucose; *2*) this impaired glucagon secretory activity cannot be due to absence of GLUT2 from α-cells because these cells do not normally express this transporter; *3*) this dysregulation may be due to inactivation of GLUT2-dependent glucose sensors located outside the endocrine pancreas and controlling glucagon secretion; and *4*) because fed hyperglucagonemia is rapidly reversed by ganglionic blockers, this suggests that in the absence of GLUT2, there is an increased activity of the autonomic nervous system stimulating glucagon secretion during the fed state. *Diabetes* 50:1282–1289, 2001

Glucagon secretion is an immediate response to glucopenia. Abnormal secretion of this and other counterregulatory hormones is a hallmark of type 1 diabetes of long duration and represents a major limitation to intensive insulin therapy (1–4). The molecular mechanisms of glucose detection triggering counterregulation and, in particular, inducing glucagon secretion, or suppressing it during hyperglycemic episodes, are not identified. There is evidence that multiple mechanisms may participate in the control of glucagon secretion, but the relative contribution of each is still debated. First, direct glucose sensing by α-cells may account for the glucagon secretory activity detected, for instance, in perfused pancreas experiments (5) or with isolated islets of Langerhans (6). However, in these conditions, hypoglycemia appears to represent a permissive condition for, and hyperglycemia may block, the secretagogue activity of other nutrients such as amino acids. That α-cells may possess a glucose sensor is nevertheless supported by their expression of molecules known to participate in glucose sensing in pancreatic β-cells: glucokinase (7) and the K_{ATP} channel subunits, SUR1 and Kir6.2 (8). However, it is important to note that, in contrast to β-cells, α-cells do not express the glucose transporter GLUT2 (9,10).

Second, the secretory activity of α-cells is also under the control of the parasympathetic and sympathetic nervous system and the sympatoadrenal axis (11,12). Activation of the autonomic nervous system (ANS) is thought to play a major role in the stimulation of glucagon secretion under hypoglycemic conditions, as determined in several species including mouse, dog, and primate (13–15). At the level of the α-cells, this stimulatory activity is mediated through activation of muscarinic, adrenergic, or peptidergic receptors. Parasympathetic nerves within the pancreas and islets have been shown to contain peptides such as cholecystokinin or gastrin-releasing peptide, and sympathetic nerves contain neuropeptide Y or galanin (12,16).

Activation of the sympathetic or parasympathetic nervous systems is controlled by different parts of the hypothalamus—mainly the ventromedial and lateral nuclei. The mechanisms by which these centers become activated by changes in glycemic levels are not completely understood at the molecular level. It has however been described that glucose-sensitive neurons are present in these different parts of the hypothalamus (17–21). These neurons respond to low- or high-glucose concentrations either by increasing or decreasing their firing rates. Their activity may also be influenced by glucose-sensitive neurons of the nucleus of

From the Institute of Pharmacology and Toxicology, University of Lausanne, Lausanne, Switzerland.

Address correspondence and reprint requests to Bernard Thorens, Institute of Pharmacology and Toxicology, University of Lausanne, 27, rue du Bugnon, CH-1005 Lausanne, Switzerland. E-mail: bthorens@ipharm.unil.ch.

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ANS, autonomic nervous system.

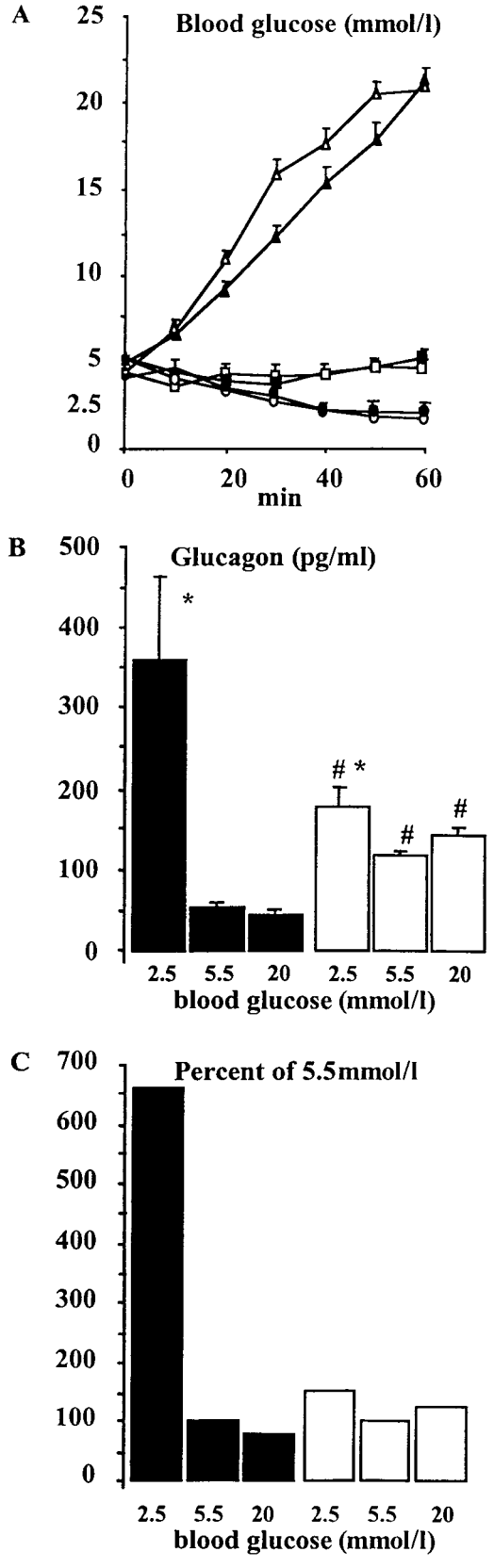

FIG. 1. Impaired glucagon response to hypoglycemia and hyperglycemia. Mice were infused with insulin at a constant rate (18 mU · kg^{-1} · min^{-1}) for 60 min and co-infused with glucose to reach the desired glycemic levels. *A*: Glycemic profiles obtained over the time course of the experiments for control and mutant mice. *B*: Plasma glucagon levels measured at the end of the 60-min infusion. *C*: Relative plasma glucagon levels at the end of the experiments. Hypoglycemia induces a strong increase in plasma glucagon in control mice and only a small increase in mutant mice. Hyperglycemia has no effect in these conditions on glucagon plasma levels. Data are means ± SE; n = 6–7 for each data point. *P < 0.05 vs. 5.5 mmol/l glucose; #P < 0.05 vs. corresponding values of control mice. ■, Control; □, RIPGLUT1 × GLUT2$^{-/-}$.

TABLE 1
Plasma insulin, glucagon, and glucose levels in control and RIPGLUT1 × GLUT2$^{-/-}$ mice in the fed state and after a 6-h fast

	Glucose (mmol/l)	Glucagon (ng/ml)	Insulin (μU/ml)
Control			
Fed	7.9 ± 0.3	61.2 ± 4.8	76.2 ± 11.4
Fasted	4.8 ± 0.1	50.6 ± 3.3	10.0 ± 2.9
RIPGLUT1 × GLUT2$^{-/-}$			
Fed	8.6 ± 0.2	108.1 ± 9.2*	17.7 ± 3.3*
Fasted	4.2 ± 0.1*	42.0 ± 3.3	2.0 ± 0.1*

Data are means ± SE. *Significantly different from control mice: P < 0.05.

the tractus solitarius, which are directly sensitive to glycemic variations and send projections toward the lateral hypothalamus and paraventricular nucleus (20,22–25). It has been demonstrated that glucose-sensitive neurons of the brainstem participate in the response of the ANS to hypoglycemia in the dog by showing that counterregulation could be partially suppressed by maintaining normoglycemia specifically in the brainstem (26).

The molecular basis of glucose sensing has been best described in the pancreatic β-cells where elevations in extracellular glucose concentrations lead to insulin granule exocytosis. This glucose-activated signaling pathway is initiated by uptake of glucose by GLUT2 and its phosphorylation by glucokinase. After glucose metabolism through the glycolytic pathway and activation of mitochondrial metabolism, coupling factors are generated that lead to membrane depolarization, entry of calcium, and insulin secretion (27,28). We have recently described the establishment of GLUT2-null mice. These mice have a severe impairment in glucose-stimulated insulin secretion, characterized by a complete loss of first-phase secretion, and die within the first 3 weeks of life if not treated with insulin (29). We have shown more recently that reexpression by transgenesis of GLUT2, or GLUT1, solely in the pancreatic β-cells completely restored glucose-stimulated insulin secretion. This allowed mouse survival to the adult age and breeding (30). These mice (RIPGLUT1 × GLUT2$^{-/-}$ or RIPGLUT2 × GLUT2$^{-/-}$) therefore represent an interesting model to study the physiological impact of GLUT2 absence from the other tissues where it is normally expressed.

Here, we studied whether glucagon secretion in response to variations in blood glucose concentrations was altered in the absence of GLUT2 outside the β-cell. This study was prompted by the observation that in the absence of GLUT2, either in the GLUT2-null mice or in the mice rescued by transgenic reexpression of GLUT1 or GLUT2 in β-cells, the fed glucagon levels were approximately twofold higher than those in control mice (30), suggesting an abnormal control of glucagon secretion. We showed that in RIPGLUT1 × GLUT2$^{-/-}$ mice, glucagonemia was no longer increased during hypoglycemic clamps or decreased during hyperglycemic clamps. More importantly, the elevated fed plasma glucagon levels could be rapidly

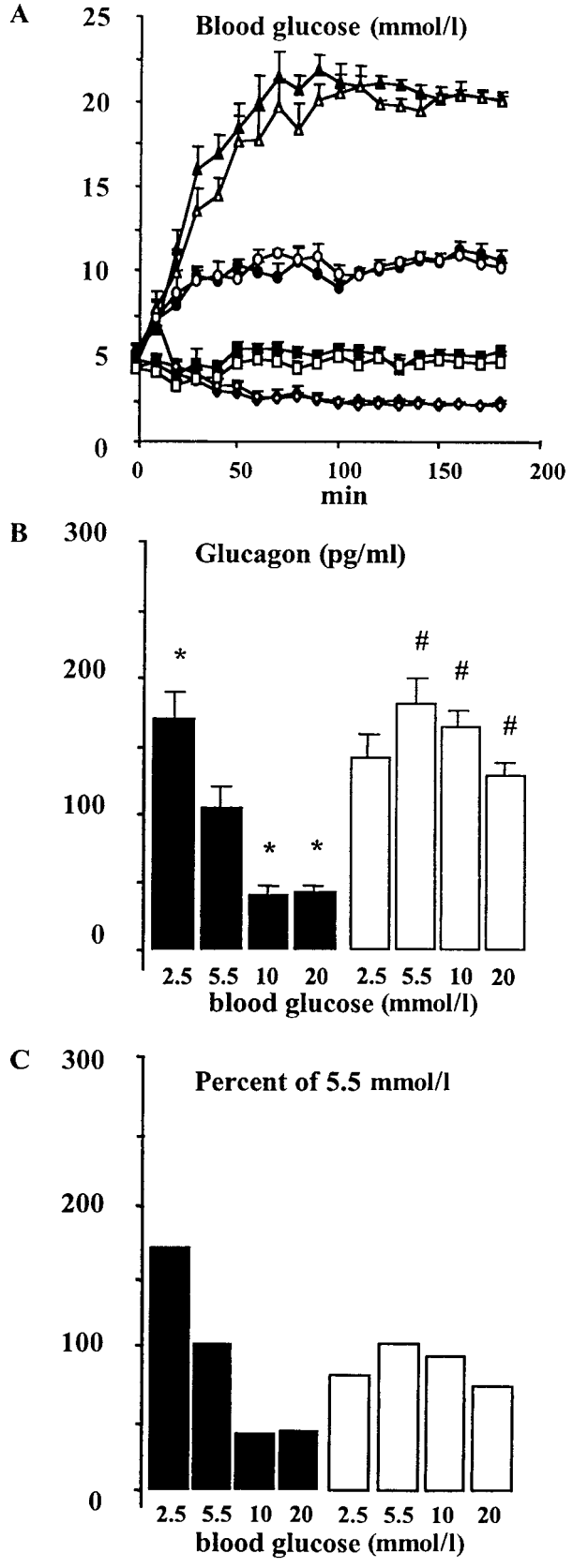

FIG. 2. Impaired glucagon response to hypoglycemic and hyperglycemic-hyperinsulinemic clamps. Mice were infused with insulin at a constant rate (18 mU · kg^{-1} · min^{-1}) for 180 min and co-infused with glucose to maintain the desired glycemic levels. *A*: Glycemic profiles obtained over the time course of the experiments for control and mutant mice. *B*: Plasma glucagon levels measured at the end of the 180-min clamps. *C*: Relative plasma glucagon levels at the end of the clamp experiments. Hypoglycemia induces a 1.7-fold increase in plasma glucagon in control mice and no increase in mutant mice. Hyperglycemia decreased plasma glucagon levels by half in control mice but had no significant effect in mutant mice. Data are means ± SE; n = 5–8 for each data point. *P < 0.05 vs. 5.5 mmol/l glucose; #P < 0.05 vs. corresponding values of control mice. ■, Control; □, RIPGLUT1 × GLUT2$^{-/-}$.

decreased to the value of the control mice by ganglionic blockers. These results provide evidence for the involvement of GLUT2 in glucose-sensing units present outside of the β-cell that control α-cell secretory activity.

RESEARCH DESIGN AND METHODS

Animals. The 10- to 14-week-old RIPGLUT1 × GLUT2$^{-/-}$, C57Bl/6, or C57Bl/6 × SV129 male mice from our colonies were used in these studies and housed with an inverted light cycle with the dark phase from 8:00 A.M. to 8:00 P.M. RIPGLUT1 × GLUT2$^{-/-}$ mice were derived from GLUT2-null mice (29) by transgenic reexpression of GLUT1 specifically in pancreatic β-cells under the control of the rat insulin promoter (30). The RIPGLUT1 × GLUT2$^{-/-}$ mice have restored normal glucose-stimulated insulin secretion as assessed in perifusion experiments and by hyperglycemic clamps (30).

Surgical procedures. An indwelling catheter was implanted in mice into the vena cava through the left femoral vein as described (31,32). The other extremity of this catheter was slid under the back skin, exteriorized, and glued at the back of the neck. The mice were then housed individually. After 2 days, they showed normal feeding behavior and motor activity.

Hyperinsulinemic clamp procedures. At 3–5 days after catheter implantations, food was removed at 8:00 A.M. for 6 h. In awake, freely moving mice, human insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was infused at the rate of 18 mU · kg^{-1} · min^{-1} for 60 or 180 min through the femoral vein catheter. A glucose solution (33% wt/vol) was co-infused with insulin to maintain blood glucose levels at the constant values of 2.5, 5.5, 10, or 20 mmol/l during the experimental period. Blood glucose concentration was monitored using a glucose meter (Roche Diagnostics, Rotkreuz, Switzerland) from 3.5 μl blood sampled approximately every 10 min. At completion of the infusions, blood was collected for plasma glucagon and insulin determinations.

Intraperitoneal injections. To block the ANS, fed mice were injected with 5 mg/kg of the ganglionic blockers hexamethonium (Sigma, St. Louis, MO) or chlorisondamine (Novartis, Summit, NJ). Blood was collected 30 min after the injections for plasma glucagon, insulin, and glucose determinations. To induce a rapid and profound hypoglycemia, 6-h fasted mice were injected with 0.7 U/kg human insulin. Blood was collected 30 or 60 min after the injection for plasma glucagon and glucose determinations. Blood glucose was monitored as described above.

Analytical procedures and determinations. Blood was collected in the presence of 1 μg/ml aprotinine and 1 mmol/l EDTA and immediately centrifuged, and the plasma was stored at –70°C until assayed. Plasma glucagon and insulin concentrations were assessed by radioimmunoassay (Linco, St. Louis, MO). Plasma glucose concentration was assessed using a glucose oxidase method (Sigma).

RESULTS

Table 1 shows the plasma glucose, glucagon, and insulin levels of control and RIPGLUT1 × GLUT2$^{-/-}$ mice in the fed and fasted states. The data show that glycemia of fed mutant mice was similar to that of control mice. In the fasted state, the mutant mice were slightly hypoglycemic, which could probably be explained by impaired renal glucose reabsorption in the absence of GLUT2. Plasma glucagon levels of the mutant mice were twice those of control mice in the fed state and were similar to those of control mice in fasted animals. Plasma insulin levels were lower in both fed and fasted states in the mutant mice.

To evaluate whether there was a defect in the control by glucose of glucagon secretion in the absence of GLUT2, we first infused mice with insulin and glucose to reach glycemic levels of 2.5, 5, or 20 mmol/l and determined the plasma glucagon levels after 60 min. Figure 1*A* shows the glycemic profiles established, and Fig. 1*B* and *C* show the absolute and relative plasma glucagon concentrations

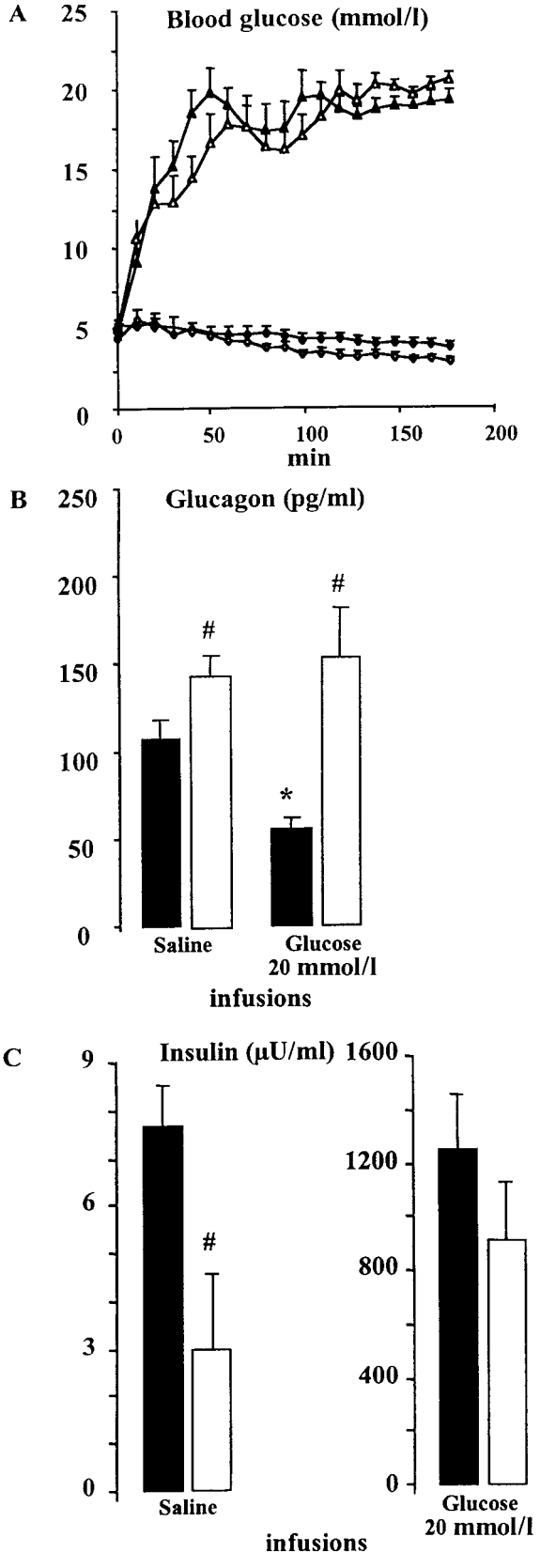

FIG. 3. Impaired glucagon response, but normal insulin secretion, during hyperglycemic clamps. Mice were infused with saline or with a glucose solution to maintain glycemia at 20 mmol/l for 3 h. At the end of the experiments, plasma glucagon and insulin were determined. *A*: Glycemic profiles maintained over the time of the experiment. *B*: Plasma glucagon at the end of the 3-h clamp experiments. *C*: Insulin levels at the end of the 3-h clamp experiments. Hyperglycemia induced an identical secretion of insulin in control and mutant mice. However, glucagon levels were not suppressed in mutant mice, indicating a lack of suppressive effect of intra-islet insulin in these experimental conditions. Data are means ± SE; $n = 5–8$ for each data point. *$P < 0.05$ vs. 5.5 mmol/l glucose; #$P < 0.05$ vs. corresponding values of control mice. ■, Control; □, RIPGLUT1 × GLUT2$^{-/-}$.

determined at the end of the infusion experiments. Hypoglycemia reached a value of ~2.0–2.5 mmol/l within ~40 min and stayed at this level until 60 min. In control mice, this hypoglycemia induced an approximately sevenfold increase in plasma glucagon levels, whereas the increase was only ~50% in the mutant mice.

Hyperglycemia developed linearly over the 60-min time course of this experiment to reach a value of 20 mmol/l, and in this condition, no significant suppression of glucagon levels could be observed in either type of mice. The lack of glucagon suppression in these conditions may be due to the relatively slow kinetics of establishment of hyperglycemia.

We next performed much longer hyperinsulinemic clamps with glucose maintained at 2.5, 5.5, 10, or 20 mmol/l for 180 min and determined plasma glucagon levels at the end of the experiments. Figure 2*A* shows the glycemic profiles that were established during the experiments. Figure 2*B* and *C* show that, in control mice, hypoglycemia increased plasma glucagon by ~1.7-fold and hyperglycemia at 10 or 20 mmol/l decreased glucagonemia by half. In contrast, in RIPGLUT1 × GLUT2$^{-/-}$ mice, no significant changes in glucagon levels occurred in response to either hypo- or hyperglycemia.

Maintaining normoglycemia during the clamp was associated with an increase in plasma glucagon levels compared with nonclamped 6 h–fasted mice (compare Fig. 2*B* and Table 1). This increase could be explained by the stress due to the experimental protocol, which requires frequent blood sampling of awake animals. A higher stress level was confirmed by elevated plasma catecholamine levels at the end of the clamp (data not shown).

In the experiments of Figs. 1 and 2, glycemic levels were maintained in the presence of continuous exogenous insulin infusion. To evaluate the role of endogenously produced insulin, we performed hyperglycemic clamps by infusing glucose in control and mutant mice to reach a glycemia of 20 mmol/l. Figure 3*A* shows the glycemic profiles established during glucose or saline infusion for 3 h, and Fig. 3*B* and *C* present the glucagon and insulin levels measured at the end of the experiment. These data show that the insulin secretory response is the same in control and mutant mice but that in these clamp conditions, there is still no suppression of glucagon secretion by hyperglycemia, even though insulin secretion is normal.

We next evaluated whether the impaired glucagon secretory activity in response to hypoglycemia was due to a complete or a relative impairment of the effect of low glucose. We thus injected control and mutant mice intraperitoneally with insulin to rapidly induce a profound hypoglycemia. As shown in Fig. 4*A*, the glycemic levels rapidly reached a value of ≤1 mmol/l. The plasma glucagon levels were then measured at 30 and 60 min and are shown in Fig. 4*B* and *C*. Under these severe hypoglycemic conditions, strong elevations in plasma glucagon levels were detected both in control (~8-fold and ~2.5-fold over basal at 30 and 60 min, respectively) and mutant mice (~5-fold and ~2-fold over basal at 30 and 60 min, respectively).

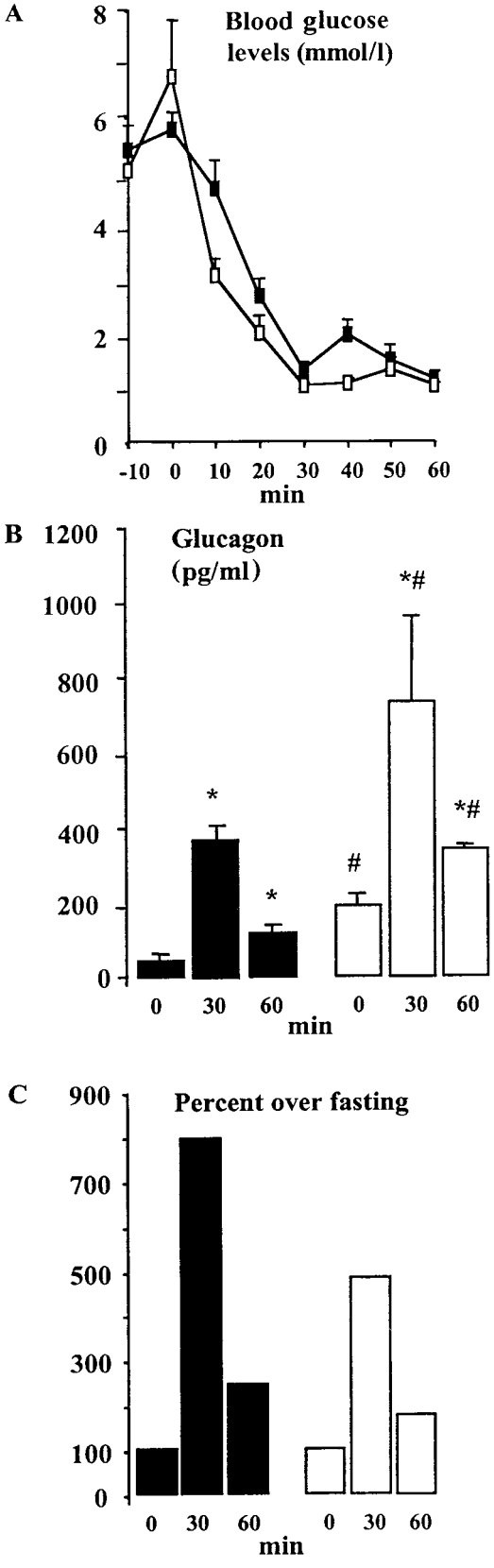

FIG. 4. Induction of severe hypoglycemia induces a strong glucagon response in both control and mutant mice. Mice were injected intraperitoneally with insulin (0.7 U/kg), and plasma glucagon was measured 30 and 60 min after the injection. *A*: Glycemic profiles obtained over the time course of the experiments for control and mutant mice. *B*: Plasma glucagon levels measured 30 and 60 min after insulin injection. *C*: Relative plasma glucagon levels 30 and 60 min after insulin injection. Hypoglycemia induced plasma glucagon both in control (~8-fold and ~2.5-fold over basal at 30 and 60 min, respectively) and mutant mice (~5-fold and ~2-fold over basal at 30 and 60 min, respectively). Data are means ± SE; $n = 6$ for each data point. *$P < 0.05$ vs. time 0; #$P < 0.05$ vs. corresponding values of control mice. ■, Control; □, RIPGLUT1 × GLUT2$^{-/-}$.

To assess whether the high plasma glucagon level found in the fed mutant mice may be due to an uncontrolled permanent stimulation of α-cells by the ANS, we injected mutant and control mice with a saline solution or a solution containing hexamethonium or chlorisondamine, two different ganglionic blockers. Plasma glucagon levels were then measured 30 min later. Figure 5*A* shows that the plasma glucagon levels were not modified in the control mice, but in RIPGLUT1 × GLUT2$^{-/-}$ mice, they were decreased to a level similar to that of control mice. Figure 5*B* and *C* show the plasma insulin and glycemic levels measured in the same mice. Injection of ganglionic blockers had a similar effect in decreasing insulin and glucose levels in control and mutant mice.

DISCUSSION

This study provides evidence that GLUT2 is required for the normal control of glucagon secretion in response to both hypo- and hyperglycemia. Because GLUT2 is not normally expressed in pancreatic α-cells, it is required for the function of glucose-sensing units controlling glucagon secretion likely located outside of the islets. Furthermore, in the absence of GLUT2, an increased ANS tone appears to be responsible for the elevated glucagon levels found in the fed state.

The GLUT2 glucose transporter is not expressed by pancreatic α-cells of normal mice (9,10) and is therefore not involved in direct glucose sensing by these cells. However, GLUT2 is required for glucose sensing by mouse pancreatic β-cells because its absence leads to loss of first-phase and diminished second-phase glucose-stimulated insulin secretion (29). Re-expression by transgenesis of GLUT1 or GLUT2 in pancreatic β-cells rescues the GLUT2-null mice from early death and normalizes both the first and second phases of glucose-induced insulin secretion, as assessed in vitro in islet perifusion experiments or in vivo during hyperglycemic clamps (30) (Fig. 3). The endocrine pancreas of these mice should therefore function normally. In this context, the low insulin levels of the RIPGLUT1 × GLUT2$^{-/-}$ mice studied here cannot be explained by a β-cell defect. It can more probably be explained by the fact that in the absence of GLUT2 from the kidney tubule, there is a strong impairment in glucose reabsorption leading to a severe glycosuria (29) and, thus, an efficient insulin-independent glucose disposal. These mice are therefore in a situation just opposite to that found in mild insulin-resistant states in which normoglycemia is maintained because of relatively high insulin levels. It is however similar to the situation found, for instance, in transgenic mice overexpressing GLUT4 in muscles where increased glucose disposal due to glucose transporter overexpression is associated with normoglycemia and lower insulinemic levels in the fasted state (33).

Here, we demonstrate that glucagon secretion was only poorly stimulated by moderate hypoglycemia and not suppressed by hyperglycemia in the mutant mice, whereas strong regulation could be observed in the control ani-

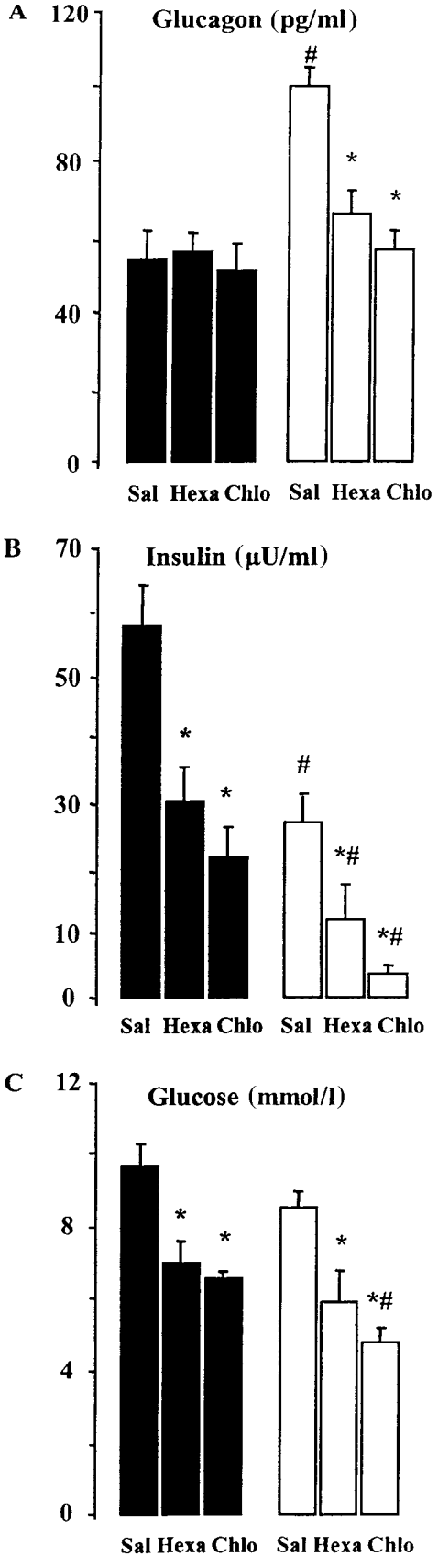

FIG. 5. Ganglionic blockade reduces plasma glucagon levels of mutant mice to the value of control mice. Fed mice were injected intraperitoneally with a saline solution (Sal) or a solution containing the ganglionic blockers hexamethonium (Hexa) or chlorisondamine (Chlo). Blood was collected for glucagon, insulin, and glucose measurements 30 min later. *A*: Ganglionic blockade decreased plasma glucagon levels in mutant mice but did not affect that of control mice. *B*: Insulin levels were decreased similarly in control and mutant mice. *C*: Plasma glucose was similarly affected in control and mutant mice. Data are means ± SE; $n = 5–6$ for each data point. *$P < 0.05$ vs. saline-injected mice; #$P < 0.05$ vs. corresponding values of control mice. ■, Control; □, RIP-GLUT1 × GLUT2$^{-/-}$.

mals. However, decreasing glycemia to ≤1 mmol/l markedly stimulated glucagon secretion in the mutant mice.

The impaired stimulation of glucagon secretion in response to moderate hypoglycemia suggests that GLUT2 expression is required for the proper detection of glucose by the sensing units controlling α-cell secretory activity. Furthermore, because normal α-cells do not express GLUT2 and the β-cells of these mice express an alternate glucose transporter, these sensing units are likely located outside of the endocrine pancreas. There is however a glucagon response to severe hypoglycemia. This response suggests a GLUT2-independent mechanism for stimulating the glucagon response to extreme hypoglycemia and indicates that the α-cells have a preserved capability to increase their secretory activity.

The preservation of a robust secretory response to severe hypoglycemia, but of only a mild one to moderate hypoglycemia, can be explained in different ways. First, there may be a single type of glucose-sensing unit controlling glucagon secretion during hypoglycemia, which is dependent on GLUT2 expression. In the absence of the transporter, a response can be stimulated only by extreme glycemic conditions. This would be similar to the secretion of insulin by GLUT2-null β-cells, which is mostly suppressed in the presence of moderate hyperglycemia but still detected with extreme glycemic levels (29,34). Second, there may be separate glucose sensors, activated at different hypoglycemic levels, that rely on different glucose transporter isoforms. It is indeed known that the counterregulatory response to hypoglycemia is graded, with the glucagon response being activated at milder hypoglycemia than the sympathoadrenal response, which, in turn, also stimulates glucagon secretion (12). In such a situation, GLUT2-dependent sensors would be activated only by moderate hypoglycemia, and severe hypoglycemia would activate another type of sensor. Third, it is possible that the increased autonomic tone to the α-cells potentiates a direct hypoglycemic stimulation of the α-cell sensor, which is prominent only at the lower glycemic levels.

In contrast to the glucagon response to hypoglycemia, suppression of glucagon secretion by hyperglycemia appears totally lost. This indicates that hyperglycemia sensing units suppressing glucagon secretion are also GLUT2-dependent. These detection units cannot be the pancreatic β-cells because their function is normal due to the transgenic reexpression of GLUT1 (30). We indeed demonstrated that high glucose normally stimulated β-cell secretory activity in hyperglycemic clamps, yet these experimental conditions did not suppress glucagon secretion.

Where are the glucose detection units located? Many studies have presented evidence for a major role of the ANS in the control of glucagon secretion during hypoglycemic episodes (35). It could therefore be hypothesized that the sensing units are located in brain regions controlling activation of the ANS (in particular the ventromedial and lateral hypothalamus). This is in agreement with the fact that GLUT2 is expressed in the hypothalamus and the

brain stem where glucose-sensitive neurons are present and where many of the proteins involved in glucose signaling in pancreatic β-cells are found. This is the case for glucokinase (36–38), the K_{ATP} channel (39–41), or the glucagon-like peptide 1 receptor (37,42,43). However, the cellular localization of GLUT2 in the hypothalamus and brain stem is not yet unambiguously established. Some reports described GLUT2 to be present in glial cells (44), whereas others found the transporters in neurons or in ependymal cells lining the third or fourth ventricle (37,38,45). Further mapping of GLUT2 expression in the central nervous system is therefore warranted.

In addition to central glucose detection units, other peripheral glucose sensors, such as those present in the hepatoportal vein, which we recently showed to be GLUT2-dependent (46,47), may also be involved in controlling ANS activity.

Although we do not yet have evidence for the location of these GLUT2-sensing units, we have shown that the hyperglucagonemia of fed mutant mice is the result of increased ANS activity. Indeed, blocking ganglionic transmission with hexamethonium or chlorisondamine rapidly led glucagon levels of mutant mice to decrease to those of fed control mice. Because the same treatment in control mice did not change their glucagon levels, this strongly suggests that in the absence of GLUT2, there is an increased tone of the ANS-stimulating glucagon secretion. Because high plasma glucagon levels are seen only in the fed state, this suggests that the ANS tone, rather than directly stimulating secretion, may sensitize α-cells to the secretagogue activity of other nutrients (e.g., amino acids). This would explain why glucagonemia returns to control levels in fasted mutant mice when the concentration of nutrients in plasma decreases. Whatever the role of the ANS tone, i.e., sensitizing or directly stimulating the α-cell secretory activity, our data suggest that GLUT2 participates in a glucose detection unit that normally controls this autonomic tone.

To explain that both hypoglycemia and hyperglycemia responses are lost in the absence of GLUT2, we propose the following hypothesis. Activity of the ANS controlling glucagon secretion may be modulated by two separate glucose sensors—one sensitive to hyperglycemia, the other sensitive to hypoglycemia, and both GLUT2-dependent. The hypoglycemia sensor continuously activates the autonomic tone, but its stimulatory activity can be suppressed by euglycemia. In the absence of GLUT2, the constant activity of the sensor can no longer be suppressed by euglycemia. In contrast, the hyperglycemia sensor, which may suppress autonomic tone, is mostly silent at euglycemia and may become activated when glucose concentrations rise. Again, in the absence of GLUT2, there is a lack of glucose sensing and therefore a lack of inhibition of the autonomic tone. Although this interpretation requires direct experimental verification, we believe that it is compatible with the glucose sensitivity of neurons found in the hypothalamus, which can respond to low- or high-glucose concentrations by increasing or decreasing their firing rates (17–21). For example, the majority of glucose-sensitive neurons of the lateral hypothalamic area decreased their firing rates in response to small increases in local glucose concentrations. In contrast, in the ventromedial hypothalamus, the majority of glucose-sensitive neurons increased their firing rates in response to small increases in local glucose concentrations (21).

Together, our data present strong evidence that extrapancreatic GLUT2-dependent glucose sensors are involved both in the stimulation of glucagon secretion in hypoglycemic conditions and in the suppression of secretion during hyperglycemia. Identification of these glucose-sensing units and the molecules they involve is of great importance not only in understanding the complexity of glucose sensing in the central nervous system, which controls diverse functions such as feeding and energy expenditure, but also because hypoglycemia unawareness and defective counterregulation are major dysfunctions associated with type 1 diabetes. Our present data point to a key role of GLUT2 in these functions.

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REFERENCES

1. Bolli G, De Feo P, Compagnucci P, Cartechini MG, Angeletti GA, Santeusanio F, Brunetti P, Gerich JE: Abnormal glucose counterregulation in insulin-dependent diabetes mellitus. *Diabetes* 32:134–141, 1983
2. White NH, Skor DA, Cryer PE, Levandoski LA, Bier DM, Santiago JV: Identification of type I diabetic patients at increased risk for hypoglycemia during intensive therapy. *N Engl J Med* 308:485–491, 1983
3. Amiel SA, Tamborlane WV, Simonson DC, Sherwin RS: Defective glucose counterregulation after strict glycemic control of insulin-dependent diabetes mellitus. *N Engl J Med* 316:1376–1383, 1987
4. Bergenstal RM, Polonsky KS, Pons G, Jaspan JB, Rubenstein AH: Lack of glucagon response to hypoglycemia in type I diabetics after long-term optimal therapy with a continuous subcutaneous insulin infusion pump. *Diabetes* 32:398–402, 1983
5. Weir GC, Knowlton SD, Martin DB: Glucagon secretion from the perfused rat pancreas: studies with glucose and catecholamines. *J Clin Invest* 54:1403–1412, 1974
6. Pipeleers DG, Schuit FC, Van Schravendijk CFH, Van De Winkel M: Interplay of nutrients and hormones in the regulation of glucagon release. *Endocrinology* 117:817–823, 1985
7. Heimberg H, De Vos A, Moens K, Quartier E, Bouwens L, Pipeleers D, Van Schaftingen E, Madsen O, Schuit F: The glucose sensor protein glucokinase is expressed in glucagon-producing a cells. *Proc Natl Acad Sci U S A* 93:7036–7041, 1996
8. Suzuki M, Fujikura K, Kotake K, Inagaki N, Seino S, Takata K: Immunolocalization of sulphonylurea receptor 1 in rat pancreas. *Diabetologia* 42:1204–1211, 1999
9. Orci L, Thorens B, Ravazzola M, Lodish HF: Localization of the pancreatic beta cell glucose transporter to specific plasma membrane domains. *Science* 245:295–297, 1989
10. Heimberg H, De Vos A, Pipeleers D, Thorens B, Schuit F: Differences in glucose transporter gene expression between rat pancreatic α- and β-cells are correlated to differences in glucose transport but not in glucose utilization. *J Biol Chem* 270:8971–8975, 1995
11. Ahren B, Taborsky GJ, Porte D: Neuropeptidergic versus cholinergic and adrenergic regulation of islet hormone secretion. *Diabetologia* 29:827–836, 1986
12. Havel PJ, Taborsky GJ: The contribution of the autonomic nervous system to changes of glucagon and insulin secretion during hypoglycemic stress. *Endocr Rev* 10:332–350, 1989
13. Havel PJ, Akpan JO, Curry DL, Stern JS, Gingerich RL, Ahren B: Autonomic control of pancreatic polypeptide and glucagon secretion during neuroglucopenia and hypoglycemia in mice. *Am J Physiol* 265:R246–R254, 1993
14. Havel PJ, Veith RC, Dunning BE, Taborsky GJ: Role for autonomic nervous system to increase pancreatic glucagon secretion during marked insulin-induced hypoglycemia in dogs. *Diabetes* 40:1107–1114, 1991
15. Havel PJ, Valverde C: Autonomic mediation of glucagon secretion during

insulin-induced hypoglycemia in rhesus monkeys. *Diabetes* 45:960–966, 1996
16. Sundler F, Böttcher G: Islet innervation, with special reference to neuropeptides. In *The Endocrine Pancreas.* Samols E, Ed. New York, Raven, 1991, p. 29–52
17. Oomura Y, Yoshimatsu H: Neural network of glucose monitoring system. *J Auton Nerv Syst* 10:359–372, 1984
18. Minami T, Oomura Y, Sugimori M: Electrophysiological properties and glucose responsiveness of guinea-pig ventromedial hypothalamic neurones in vitro. *J Physiol* 380:127–143, 1986
19. Oomura Y, Ooyama H, Sugimori M, Nakamura T, Yamada Y: Glucose inhibition of the glucose-sensitive neurone in the rat lateral hypothalamus. *Nature* 247:284–286, 1974
20. Himmi T, Boyer A, Orsini JC: Changes in lateral hypothalamic neuronal activity accompanying hyper- and hypoglycemias. *Physiol Behav* 44:347–354, 1988
21. Silver IA, Erecinska M: Glucose-induced intracellular ion changes in sugar-sensitive hypothalamic neurons. *J Neurophysiol* 79:1733–1745, 1998
22. Orsini J-C, Himmi T, Wiser AK, Perrin J: Local versus indirect action of glucose on the lateral hypothalamic neurons sensitive to glycemic level. *Brain Res Bull* 25:49–53, 1990
23. Pierret P, Haddjeri N, Boyer A, Perrin J, Orsini JC: β2-adrenoceptors are involved in lateral hypothalamic unit responses to hyperglycemia. *J Physiol* 88:89–90, 1994
24. Orsini J-C, Wiser AK, Himmi T, Boyer A, Perrin J: Sensitivity of lateral hypothalamic neurons to glycemic level: possible involvement of an indirect adrenergic mechanism. *Brain Res Bull* 26:473–478, 1991
25. Yettefti K, Orsini JC, El Ouazzani T, Himmi T, Boyer A, Perrin J: Sensitivity of nucleus tractus solitarius neurons to induced moderate hyperglycemia, with special reference to catecholaminergic regions. *J Auton Nerv Syst* 51:191–197, 1995
26. Frizzell RT, Jones EM, Davis SN, Biggers DW, Myers SR, Connolly CC, Neal DW, Jaspan JB, Cherrington AD: Counterregulation during hypoglycemia is directed by widespread brain regions. *Diabetes* 42:1253–1261, 1993
27. Matschinsky FM: A lesson in metabolic regulation inspired by the glucokinase sensor paradigm. *Diabetes* 45:223–241, 1996
28. Maechler P, Wollheim CB: Mitochondrial glutamate acts as a messenger in glucose-induced insulin exocytosis. *Nature* 402:685–689, 1999
29. Guillam MT, Hümmler E, Schaerer E, Yeh J-Y, Birnbaum MJ, Beermann F, Schmidt A, Dériaz N, Thorens B: Early diabetes and abnormal postnatal pancreatic islet development in mice lacking Glut-2. *Nat Genet* 17:327–330, 1997
30. Thorens B, Guillam M-T, Beermann F, Burcelin R, Jaquet M: Transgenic reexpression of Glut1 or Glut2 in pancreatic β cells rescues Glut2-null mice from early death and restores normal glucose-stimulated insulin secretion. *J Biol Chem* 275:23751–23758, 2000
31. Kamohara R, Burcelin R, Halaas JL, Friedman JM, Charron MJ: Acute stimulation of glucose metabolism in mice by leptin treatment. *Nature* 389:374–377, 1997
32. Guillam M-T, Burcelin R, Thorens B: Normal hepatic glucose production in the absence of GLUT2 reveals an alternative pathway for glucose release from hepatocytes. *Proc Natl Acad Sci U S A* 95:12317–12321, 1998
33. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CMB, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JPH, Smith SM, Ghatei MA, Herbert J, Bloom SR: A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 379:69–72, 1996
34. Guillam M-T, Dupraz P, Thorens B: Glucose uptake, utilization, and signaling in Glut2-null islets. *Diabetes* 49:1485–1491, 2000
35. Taborsky GJ, Ahrén B, Havel PJ: Autonomic mediation of glucagon secretion during hypoglycemia: implication for impaired alpha cell responses in type 1 diabetes. *Diabetes* 47:995–1005, 1998
36. Yang XJ, Kow LM, Funabashi T, Mobbs CV: Hypothalamic glucose sensor: similarities to and differences from pancreatic β-cell mechanisms. *Diabetes* 48:1763–1772, 1999
37. Navarro M, Rodriguez de Fonseca F, Alvarez E, Chowen JA, Zueco JA, Gomez R, Eng J, Blazquez E: Colocalization of glucagon-like peptide-1 (GLP-1) receptors, glucose transporter GLUT2, and glucokinase mRNAs in rat hypothalamic cells: evidence for a role of GLP-1 receptor agonist as an inhibitory signal for food and water intake. *J Neurochem* 67:1982–1991, 1996
38. Jetton TL, Liang Y, Pettepher CC, Zimmerman EC, Cox FG, Horvath K, Matschinsky FM, Magnuson MA: Analysis of upstream glucokinase promoter activity in transgenic mice and identification of glucokinase in rare neuroendocrine cells in the brain and gut. *J Biol Chem* 269:3641–3654, 1994
39. Ashford ML, Boden PR, Treherne JM: Glucose-induced excitation of hypothalamic neurones is mediated by ATP-sensitive K+ channels. *Pflügers Arch* 415:479–483, 1990
40. Lee K, Dixon AK, Richardson PJ, Pinnock RD: Glucose-receptive neurones in the rat ventromedial hypothalamus express KATP channels composed of Kir6.1 and SUR1 subunits. *J Physiol* 515:439–452, 1999
41. Sakura H, Trapp S, Liss B, Ashcroft FM: Altered functional properties of KATP channels conferred by a novel splice variant of SUR1. *J Physiol* 521:337–350, 1999
42. Shughrue PJ, Lane MV, Merchenthaler I: Glucagon-like peptide-1 receptor (GLP1-R) mRNA in the rat hypothalamus. *Endocrinology* 137:5159–5162, 1996
43. Ørskov C, Poulsen SS, Møller M, Holst JJ: Glucagon-like peptide 1 receptors in the subfornical organ and the area postrema are accessible to circulating glucagon-like peptide 1. *Diabetes* 45:832–835, 1996
44. Leloup C, Arluison M, Lepetit N, Cartier N, Marfaing-Jallat P, Ferré P, Pénicaud L: Glucose transporter 2 (GLUT2): expression in specific brain nuclei. *Brain Res* 638:221–226, 1994
45. Maekawa F, Toyoda Y, Torii N, Miwa I, Thompson RC, Foster DL, Tsukahara S, Tsukamura H, Maeda K-I: Localization of glucokinase-like immunoreactivity in the rat lower brain stem: for possible location of brain glucose-sensing mechanisms. *Endocrinology* 141:375–384, 2000
46. Burcelin R, Dolci W, Thorens B: Portal glucose infusion in the mouse induces hypoglycemia: evidence that the hepatoportal glucose sensor stimulates glucose utilization. *Diabetes* 49:1635–1642, 2000
47. Burcelin R, Dolci W, Thorens B: Glucose sensing by the hepatoportal sensor is GLUT2-dependent: in vivo analysis in GLUT2-null mice. *Diabetes* 49:1643–1648, 2000