Rapid translocation of hepatic glucokinase in response to intraduodenal glucose infusion and changes in plasma glucose and insulin in conscious rats

Chang An Chu, Yuka Fujimoto, Kayano Igawa, Joseph Grimsby, Joseph F. Grippi, Mark A. Magnuson, Alan D. Cherrington, and Masakazu Shioita

Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0615; and Department of Metabolic Diseases, Hoffmann-La Roche, Nutley, New Jersey 07110

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The liver plays a major role in glucose homeostasis by switching its intermediary metabolism from glucose production to glucose uptake in response to changes in nutritional status. Felig et al. (12) demonstrated that oral intake of glucose in starved men switched the splanchic carbohydrate balance from glucose production to glucose utilization within minutes. In fasted conscious dogs, net hepatic glucose flux switched from output to uptake within 15 min after intraduodenal infusion of glucose (26, 39). Similarly, increasing plasma glucose and insulin levels with peripheral infusion of glucose and intraportal infusion of insulin switched net hepatic glucose flux from production to uptake within 15 min (27). Such rapid switching has been proposed to be mediated by increased glucose delivery to the liver and/or sinusoidal blood glucose concentrations, increased plasma insulin concentrations, and a signal prompted by the occurrence of a negative hepatic arterial-portal difference in glucose concentration (33). However, the intracellular mechanism by which these factors trigger net hepatic glucose uptake so rapidly has not been completely elucidated.

The net hepatic glucose flux is the balance between the rate of glucose phosphorylation catalyzed by glucokinase (GK) and the rate of dephosphorylation of glucose-6-phosphate catalyzed by glucose-6-phosphatase. Van Schaftingen et al. (47) demonstrated that GK activity is acutely regulated by its interaction with its regulatory protein (GKRP). GKRP binds to GK and allosterically inhibits the enzyme by decreasing the apparent affinity of the enzyme for glucose. Fructose-1-phosphate, an intracellular metabolite, stimulates the dissociation of GK from GKRP by decreasing the binding affinity of GKRP with GK (8). Studies using isolated hepatocytes have shown that catalytic amounts of fructose or sorbitol, a precursor of fructose-1-phosphate, markedly increase the rate of glucose phosphorylation (1, 8, 44) by stimulating GK dissociation from GKRP and GK export from the nucleus (7, 20, 44). Recently, we showed that intraportal and intraduodenal infusions of a small amount of fructose markedly increased net hepatic glucose uptake in conscious dogs (37, 39). Translocation of GKRP, on the other hand, has not been detected (7, 20) or has been reported to occur partially, if at all, in response to high concentrations of glucose (30, 43), fructose (43), or sorbitol (30). These findings indicate that the dissociation of GK from GKRP and subsequent GK translocation from the nucleus to the cytoplasm have a large impact on glucose phosphorylation and glucose uptake in the liver.

If GK translocation is the primary mechanism by which the liver switches from production to uptake, it should be regulated by major physiological mediators of net hepatic glucose balance, it should occur rapidly after feeding or in response to increased blood glucose, and it should exhibit a time course that coincides with changes in the hepatic glucose balance in vivo. Glucose has been shown to be a major factor in regulating net hepatic glucose flux by suppressing glucose production and by stimulating glucose uptake (4, 33, 35). Acius and Peak...
Logical mediators for switching net hepatic glucose feeding. Glucose balance from production to uptake in response to results from in vitro studies lead us to hypothesize that GK stimulates GK translocation in the presence of glucose in Peak (1) and Toyoda et al. (43) have reported that insulin mediator in regulating hepatic glucose balance from production to uptake in vivo. The half-maximal responses of GK release (1, 30) or GK translocation (44) was observed 5–10 min after the rise in glucose. This time course correlates well with the time course of the switch in hepatic glucose balance from production to uptake in vivo. The rise in plasma insulin has also been shown to be a major mediator in regulating hepatic glucose flux (5, 33). Agius and Peak (1) and Toyoda et al. (43) have reported that insulin stimulates GK translocation in the presence of glucose in cultured hepatocytes or perfused liver, respectively, whereas Brown et al. (7) did not observe such an effect of insulin. These results from in vitro studies lead us to hypothesize that GK translocation plays a physiological role in switching hepatic glucose balance from production to uptake in response to feeding.

To further test this hypothesis, we examined major physiological mediators for switching net hepatic glucose flux in conscious rats. First, we asked whether in response to intraduodenal glucose infusion, GK translocation takes place at a time course (within minutes) consistent with the switch in net hepatic glucose balance seen after an oral glucose load. Second, we asked whether increasing plasma glucose and/or insulin levels in the physiological range could induce GK translocation.

**MATERIALS AND METHODS**

**Animals and Surgical Methods**

Male Sprague-Dawley rats, weighing 280–340 g, were used. Surgery was performed 2 wk before each experiment. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip). The right external jugular vein and the left common carotid artery were cannulated with sterile silicone catheters [0.64 mm inner diameter (ID)/1.19 mm outer diameter (OD) and 0.51 mm ID/0.94 mm OD, respectively]. After a midline laparotomy from the xyphoid process to the pubic bone, silicone catheters (0.64 mm ID/1.19 mm OD and 0.51 mm ID/0.94 mm OD) were placed in the hepatic portal vein and the ileal vein, respectively. A gastric cannula made of silicone tubing (0.64 mm ID/1.19 mm OD) was passed through and tied to the stomach wall. It was extended ~10 mm into the duodenum. In the studies measuring hepatic blood flow, some rats did not have the ileal and hepatic portal catheters. A catheter (0.51 mm ID/0.94 mm OD) was placed in the right femoral artery for reference blood samples. The free ends of these catheters were passed subcutaneously to the back of the neck where they were fixed. Catheters were occluded with metal plugs following a flush of heparinized saline solution (200 U heparin/ml saline). The success rate of the surgery and catheterization procedures was ~80%. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, and all protocols were approved by the Vanderbilt University Institutional Animal Care and Use Committee.

**Experimental Design**

Each experiment was conducted on a conscious rat that had been fasted for 6 h. In the first part of the study, the effect of the portal and ileal vein catheterization on hepatic arterial and portal blood flows was determined in rats with and without the indwelling catheters in the ileal vein and hepatic portal vein, according to Malik et al. (24). Additionally, the catheters in the carotid artery, jugular vein, stomach, and femoral artery were also examined. A volume of 0.2 ml containing 50,000 microspheres (15 μm diameter, 109 Nb and 113 Sn were injected, respectively. It has been reported that injection of 15-μm microspheres at the used dose produces no change in blood pressure (24) and that sequential injection of 15-μm microspheres does not affect the results of the consecutive determinations with the second or third set of injected microspheres (40, 41, 45). At the end of the experiments, the animals were killed by intravenous injection of pentobarbital sodium (100 mg/kg). Liver, stomach, intestine, spleen, mesentery, and kidney were dissected and weighed. The organs were cut into pieces and transferred into counting tubes. The total radioactivity in all organs was determined. The radioactivities of the reference blood, organ, and tissue samples were measured in a gamma counter, and the blood flow was calculated according to Malik et al. (24). Flows in the portal vein could not be directly measured, because microspheres are trapped in the intestines (and in the liver via the hepatic artery) (41). The flow in the portal vein, therefore, was calculated as the sum of arterial flow to the stomach, small and large intestines, mesentery, and spleen.

In the second part of the study, the effect of intraduodenal glucose infusion was determined. After a 90-min equilibration period (~120 to ~30 min) and a 30-min control period (~30 to 0 min), there was a 120-min test period. During the latter, glucose was given at 500 mg/kg as bolus, after which it was infused at 28 mg·kg⁻¹·min⁻¹. At 60 and 120 min after the start of the glucose infusion, microspheres (15 μm) labeled with 99Nb and 113Sn were injected, respectively. It has been reported that injection of 15-μm microspheres at the used dose produces no change in blood pressure (24) and that sequential injection of 15-μm microspheres does not affect the results of the consecutive determinations with the second or third set of injected microspheres (40, 41, 45). Each study consisted of a 120-min equilibration period (~120 to ~30 min) and a 30-min control period (~30 to 0 min), there was a 120-min test period. During the latter, glucose was given at 500 mg/kg as bolus after which it was infused at 28 mg·kg⁻¹·min⁻¹. Blood samples (0.25 ml) were taken from arterial and hepatic portal vein catheters before and at 5, 10, 20, 30, and 120 min during the test period. The total volume of sampled blood was 2.5 ml, which was ~12% of the animal’s total blood volume. Some animals (n = 5 at each time point) were anesthetized by intravenous injection of pentobarbital sodium (40 mg/kg) after blood sampling at 0, 10, 20, 30, and 120 min, and a laparotomy was immediately performed. The median lobe of the liver was excised and dropped into fixation solution for immunohistochemical assay. The left lobe of the liver was frozen in situ using Wollenberg tongs precooled in liquid nitrogen. This procedure took <20 s from the point of successful anesthesia.

In the third part of the study, we determined whether the translocation of GK in response to intraduodenal glucose infusion was induced by hyperglycemia, hyperinsulinemia, or both. To do so, four protocols (n = 5 each) were performed on 6-h-fasted conscious rats. Each study consisted of a 120-min equilibration period (~120 to 0 min) and a 30-min clamp period (0–30 min). Starting at ~120 min, somatostatin (2 μg·kg⁻¹·min⁻¹) was infused peripherally to inhibit endogenous insulin and glucagon secretion. Concurrently, insulin (1 mU·kg⁻¹·min⁻¹) and glucagon (2 ng·kg⁻¹·min⁻¹) were infused into the hepatic portal vein through the ileal catheter to maintain basal hormone levels. Glucose was infused through the jugular vein catheter as required. During the clamp period (0–30 min), blood glucose and plasma insulin levels were kept basal (group 1), the blood glucose level was raised to 170 mg/dl in the presence of basal insulin (group 2), or the insulin infusion rate was increased to 15 mU·kg⁻¹·min⁻¹ to raise plasma insulin levels to the range of 20–25 ng/ml and blood flow...
Table 1. Body weight and daily food intake before and after surgery

<table>
<thead>
<tr>
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<th>Before</th>
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<tr>
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<td>Body wt., g</td>
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<td>324±7</td>
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<td>2±1</td>
<td>8±1</td>
<td>19±1</td>
<td>23±2</td>
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Values are means ± SE of 25 animals.

Analytical Methods

GK and GKRP immunostaining. Livers (the median lobe) were quickly excised, rinsed in ice-cold PBS, and diced into cubes. The cubes were immediately immersed in 4% paraformaldehyde/PBS on ice and fixed for 3 h. After being washed and embedded in conventional paraffin, two serial sections (4 μm) were mounted on the same slide glass. After being deparaffinized, hydrated, and blocked in 10% normal donkey serum/PBS for several hours, an equivolume mixture of sheep anti-rat glutathione-S-transferase (GST)-GST serum and rabbit anti-rat GST-GKRP serum (1:1,000 in 5% normal donkey serum + 0.1% Triton X-100/PBS) was dropped on one of the sections. An equivolume mixture of preimmune sheep and rabbit serum (diluted 1:1,000 in 5% normal donkey serum/PBS) was dropped on the other section. Slides were kept in a humid chamber for 12 h at 4°C. After four 5-min washes in 0.1% Triton X-100/PBS, slides were incubated with Cy3-conjugated donkey anti-sheep IgG (diluted 1:1,000) and Cy5-conjugated donkey anti-rabbit IgG (diluted 1:500) as well as YoPro-1 (diluted 1:1,000) in 5% normal donkey serum/PBS for 1 h at room temperature. After being washed, sections were mounted in Aqua-PolyMount.

The liver was excised from 6-h-fastened rats in the presence of euglycemia and euninsulinemia, and the sections, made exactly as described above, were stocked. These sections were stained with the same solutions as a standard sample at each experimental time point.

Quantitative confocal microscopy. A Zeiss laser-scanning confocal microscope (LSM410) was used to record confocal images of GK and GKRP immunofluorescence patterns in liver sections. The internal argon-krypton laser was used at 568, 647, and 488 nm to optimally excite Cy3, Cy5, and YoPro fluorescence, respectively. After the transfer of image files to a Power Macintosh Imaging workstation, the image was converted to TIFF and the individual cells were quantitated using nuclear/cytoplasmic pixel density ratioing with National Institutes of Health Image (v1.56).

Table 2. Renal and hepatic blood flow in rats with and without the ileal and hepatic portal catheters before and during intraduodenal glucose infusion

<table>
<thead>
<tr>
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<th>0</th>
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<tr>
<td>Right kidney</td>
<td>Presence</td>
<td>25.3±1.4</td>
<td>29.6±2.5</td>
<td>28.8±1.4</td>
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<tr>
<td>Left kidney</td>
<td>Presence</td>
<td>25.5±1.1</td>
<td>26.0±1.6</td>
<td>29.2±1.4</td>
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<tr>
<td>Hepatic artery</td>
<td>Presence</td>
<td>24.5±1.7</td>
<td>28.8±1.7</td>
<td>27.8±1.6</td>
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<tr>
<td>Hepatic portal vein</td>
<td>Presence</td>
<td>26.8±1.6</td>
<td>26.4±1.3</td>
<td>28.2±1.2</td>
</tr>
<tr>
<td></td>
<td>Absence</td>
<td>5.2±0.9</td>
<td>4.6±0.7</td>
<td>4.9±2.3</td>
</tr>
<tr>
<td></td>
<td>Absence</td>
<td>7.5±1.4</td>
<td>5.3±1.5</td>
<td>5.5±1.5</td>
</tr>
<tr>
<td></td>
<td>Presence</td>
<td>75.7±5.2</td>
<td>93.5±5.3*</td>
<td>91.1±6.5*</td>
</tr>
<tr>
<td></td>
<td>Absence</td>
<td>72.8±3.6</td>
<td>92.8±6.9*</td>
<td>89.9±5.2*</td>
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Values are means ± SE of 5 experiments (in ml·min⁻¹·kg body wt⁻¹). Time 0 is before glucose loading. *Significantly different from before glucose loading (P < 0.05).
samples, the microspheres must be homogeneously mixed with the blood. The approximation of measured blood flow between the right and left kidney has been used as the index of adequate mixing of infused microspheres with blood (24). The blood flow was similar in the right and left kidney at all time points. There was no significant difference in hepatic arterial and portal blood flow between rats with and without the ileal and hepatic portal vein catheterizations. These blood flow values are in the range of those reported by others (23, 24, 29, 34, 46). Furthermore, hepatic portal blood flow increased in response to intraduodenal glucose infusion at increments that were similar between rats with and without the ileal and hepatic portal vein catheterizations. Therefore, the indwelling catheters in the ileal and hepatic portal vein did not affect hepatic portal and arterial blood flow.

Basal arterial and hepatic portal vein blood glucose, plasma insulin, and plasma glucagon levels were 90 ± 3 and 87 ± 3 mg/dl, 0.7 ± 0.1 and 1.8 ± 0.3 ng/ml, and 68 ± 6 and 72 ± 8 pg/ml, respectively, as shown in Fig. 1. Intraduodenal glucose infusion elevated blood glucose levels in the artery and hepatic portal at 10 min, after which blood glucose levels decreased gradually until 120 min. Plasma insulin levels in the artery and hepatic portal vein rose steadily for 20 min, after which they gradually decreased. On the other hand, plasma glucagon levels in the artery fell through 120 min.

Livers were fixed before and at 10, 20, 30, and 120 min after the start of the intraduodenal glucose infusion, followed by processing for laser confocal immunofluorescence detection of GK and GKRP. As shown in Fig. 2A, GK and GKRP immunostaining were predominantly detected in the hepatocyte nuclei before glucose infusion. After 30 min of glucose infusion, GK immunofluorescence in the nuclei decreased, whereas that in the cytoplasm increased. However, GKRP immunofluorescence remained in the nucleus. To quantify the extent of the translocation, the ratios of nuclear to cytoplasmic GK and GKRP were calculated. The ratio of nuclear to cytoplasmic GK immunofluorescence decreased through 30 min; thereafter, the ratio did not change further (Fig. 2C). These changes in GK distribution were not due to variations in the total amount of GK, because the amounts of GK protein measured by Western blot analysis using the same antibody did not differ among the different time points (1.00 ± 0.05 at 0 min, 1.10 ± 0.08 at 10 min, 0.96 ± 0.12 at 20 min, 1.05 ± 0.13 at 30 min, and 1.04 ± 0.11 at 120 min as normalized to the average value at 0 min). The intracellular distribution of GKRP immunofluorescence did not change following intraduodenal glucose infusion (Fig. 2C). The total amount of GKRP determined by immunoblotting analysis in the liver also did not change during glucose infusion (1.00 ± 0.11 at 0 min, 0.94 ± 0.17 at 10 min, 1.08 ± 0.11 at 20 min, 1.12 ± 0.13 at 30 min, and 1.05 ± 0.10 at 120 min as normalized to the average value at 0 min).

To assess the effect of rising plasma glucose and/or insulin on GK translocation, euglycemic-euinsulinemic, hyperglycemic-euinsulinemic, euhyperglycemia-euinsulinemic, or hyperglycemic-hyperinsulinemic clamps were performed for 30 min. In the euglycemic-euinsulinemic clamp (group 1), as shown in Fig. 3, arterial blood glucose and arterial and portal plasma insulin were kept at basal levels (93 ± 7 mg/dl and 0.7 ± 0.1 and 1.6 ± 0.7 ng/ml, respectively) throughout the study. In the hyperglycemic-euinsulinemic clamp (group 2), blood glucose was raised to 175 ± 5 mg/dl in the presence of basal plasma insulin levels (1.0 ± 0.3 mg/ml in artery and 2.4 ± 0.2 mg/ml in portal vein). In the euglycemic-hyperinsulinemic clamp
translocation in response to glucose infusion or changes in glucagon levels were clamped at a basal level in all groups. Levels seen in the second and third groups, respectively. The plasma insulin levels were matched to the insulin and glucose cemic-hyperinsulinemic clamp (group 4) of basal blood glucose levels (8.1–11.0), arterial and portal plasma insulin levels were raised group 3 (during 30-min clamp period. Data are the means in 6-h-fasted conscious rats. Data from each group are the average of the results at 10, 20, and 30 min during the combination of euglycemia and euinsulinemia (group 1), hyperglycemia and euinsulinemia (group 2), euglycemia and hyperinsulinemia (group 3), or hyperglycemia and hyperinsulinemia (group 4) in 6-h-fasted conscious rats. Data from each group are the average of the results at 10, 20, and 30 min.

At the end of the 30-min clamp period, livers were fixed for analysis. As shown in Fig. 4, in the euglycemic-euinsulinemic clamp (group 1), GK immunofluorescence N/C was 3.0 ± 0.5. The ratio was significantly lower under hyperglycemic-euinsulinemia (group 2: 1.5 ± 0.2, P < 0.05), euglycemic-hyperinsulinemia (group 3: 1.8 ± 0.1, P < 0.05), and hyperglycemic-hyperinsulinemia (group 4: 1.3 ± 0.1, P < 0.05). GKRP immunofluorescence N/C were very similar among all clamps (13.2 ± 2.1 in euglycemic-euinsulinemia, 10.8 ± 2.8 in hyperglycemic-euinsulinemia, 11.0 ± 2.3 in euglycemic-hyperinsulinemia, and 10.5 ± 1.8 in hyperglycemic-hyperinsulinemia). The amounts of GK and GKRP protein determined by Western blot analysis using the same anti-GK and GKRP serum were similar among all conditions (data not shown). Therefore, the changes in the intracellular distribution of GK immunoreactivity by hyperglycemia and/or hyperinsulinemia were not due to variations in the total amount of GK.

DISCUSSION

Few studies, to date, have examined the time course of GK translocation in response to glucose infusion or changes in blood glucose and/or insulin in the physiological range in vivo. Previous in vivo studies have shown a decrease in N/C GK immunoreactivity 1 h after refeeding in 24 h-fasted rats (13) and decreased GK immunofluorescence in the nucleus 30 min after oral glucose loading (44). We demonstrated here that the half-maximal response of GK translocation took place within 10 min, and the redistribution was terminated by 20–30 min after intraduodenal glucose loading in conscious rats. It has been reported in humans (12) and dogs (26, 39) that the switching of hepatic intermediary metabolism from glucose production to glucose use takes place within minutes following oral intake of glucose, intraduodenal glucose infusion, or by concurrently increasing plasma glucose and insulin levels by venous infusion. The present study demonstrates that GK translocation can take place in vivo at a rate consistent with the switch in net hepatic glucose balance seen after an oral glucose load. In our previous studies using conscious dogs, the half-maximal responses of increased net hepatic glucose uptake following intraportal infusion of small amounts of fructose, which dissociates GK from GKRP and causes translocation of GK from the nucleus to the cytoplasm (1, 8), were observed after 10 min (37). This suggests that the activation of GK by dissociating from GKRP and by transporting to the cytoplasm could cause the increase in hepatic glucose uptake. Therefore, GK translocation rates observed in response to intraduodenal glucose infusion may be rapid enough to support its contribution to the immediate switching of net hepatic glucose balance from production to uptake after a meal reported in humans (12) and animals (26, 39).

The rise in plasma glucose concentration is one of the physiological factors that mediates the switch in net hepatic glucose flux (4, 33, 35). It has been shown that the dissociation of GK from the intracellular binding site and the translocation of GK from the nucleus to the cytoplasm increases dose-dependently in the range of 5 and 20 mM glucose concentration even in the absence of insulin in cultured hepatocytes (1, 37).

Fig. 3. Blood glucose, plasma insulin, and plasma glucagon concentrations during the combination of euglycemia and euinsulinemia (group 1), hyperglycemia and euinsulinemia (group 2), euglycemia and hyperinsulinemia (group 3), or hyperglycemia and hyperinsulinemia (group 4) in 6-h-fasted conscious rats. Data from each group are the average of the results at 10, 20, and 30 min during 30-min clamp period. Data are the means ± SE from 5 experiments.

Fig. 4. The N/C immunoreactivity of GK and GKRP in the livers of rats exposed to the combination of groups 1, 2, 3, or 4. Data are means ± SE from 5 experiments. *P < 0.05 compared with N/C of GK in group 1.
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7, 44). The half-maximal response was observed at \( \sim 10-15 \) mM glucose (1, 44). In the present study, during the first 10 min of intraduodenal glucose infusion, when the GK translocation was marked, blood glucose levels in the hepatic portal vein rose from 4.8 to 11.5 mM (Fig. 2). Such a rise in blood glucose in portal blood could induce the translocation of GK in the absence of a rise in plasma insulin levels (Fig. 4). It is likely, therefore, that the immediate translocation of GK, which followed the intraduodenal glucose infusion, was mediated by the rise in plasma glucose levels.

The rise in the plasma insulin concentration is another physiological signal that mediates the switch in hepatic glucose balance (4, 17, 33). Few studies have examined the effect of insulin on GK translocation in the liver in vitro, with controversial results. Agius and Peak (1) showed that the exposure of cultured hepatocytes to 10 nM insulin increases digitonin-permeabilization-induced release of GK in the presence of 5–25 mM glucose. Toyoda et al. (42) reported that 10 nM insulin potentiated the effect of 20 mM glucose to induce GK translocation in perfused rat liver. These data suggest that insulin stimulates GK translocation and/or GK dissociation from its intracellular binding site at least in the presence of glucose. On the contrary, Brown et al. (7) reported a lack of effect by insulin on GK translocation in cultured hepatocytes using immunohistochemical techniques. So far, it is not known whether a rise in plasma insulin within physiological ranges is able to induce GK translocation in the absence of a rise in plasma glucose in vivo, because raising plasma insulin by glucose load has always been accompanied by elevated plasma glucose levels (13, 44). In the present study, plasma insulin levels in the portal vein were markedly raised from its basal levels (0.7 ng/ml) to 23 ng/ml within 10 min after the start of intraduodenal glucose infusion (Fig. 1). The rise in plasma insulin concentration to 20–25 ng/ml (similar to that seen in the hepatic portal vein after intraduodenal glucose infusion) was able to cause GK translocation in the absence of an increase in plasma glucose levels in conscious rats at least within 30 min (Fig. 4). It is likely, therefore, that the rise in plasma insulin contributes to the immediate translocation of GK after a meal.

The negative arterial-hepatic portal difference in blood glucose levels, the “portal signal,” has been reported to augment net hepatic glucose uptake (33). The effect of the portal signal reaches its maximum with an arterial-hepatic portal difference of 20–30 mg/dl in plasma glucose levels in conscious dogs (33). As shown in Fig. 1, an arterial-hepatic portal difference of \( \sim 20–30 \) mg/dl was already present at the early phase of intraduodenal glucose infusion in the present study. Although it has not been clearly shown whether the portal signal operates in rodents, it would be interesting to examine the effect of the portal signal on GK distribution. In the present study, however, we did not measure net hepatic balance because of the difficulty in sampling hepatic venous blood. Thus we cannot demonstrate whether the portal signal stimulates hepatic glucose uptake or GK translocation in this species.

The quantitative impact of GK translocation from the nucleus to the cytoplasm in increasing the phosphorylation of glucose in response to the rise in blood glucose is unclear. The immunoreactivity reflects a density (concentration) of GK and GKRP in each compartment of the cytoplasm and the nucleus. On the other hand, the volumes of the nucleus and the cytoplasm occupied in the hepatocyte are markedly different (the nuclear volume is only 10% of the cell volume). With immunohistochemical techniques, therefore, it is difficult to determine the amount of GK distributed in each compartment of the cytoplasm and the nucleus. Glucose cycling (glucose→glucose-6-phosphate→glucose) has been demonstrated in the liver under basal conditions in conscious rats (30), implying that some fraction of GK exists to phosphorylate glucose and that this GK contributes to the increase in glucose phosphorylation in response to an increased glucose mass. However, in vitro studies using isolated hepatocytes have reported that glucose phosphorylation is markedly increased by small amounts of fructose (1, 8, 44), which stimulate the dissociation of GK from GKRP and the translocation of GK (7, 20, 44). We have shown previously (37) that in a conscious dog, a catalytic amount of fructose causes a three-fold increase in net hepatic glucose uptake and subsequent glycogen synthesis from glucose and glycolysis even in the presence of hyperglycemic hyperinsulinemia. This suggests an impact on hepatic glucose phosphorylation in vivo by the dissociation of GK from GKRP. Whereas the stimulation of GK dissociation from GKRP is normally accompanied by GK translocation from the nucleus to the cytoplasm, it remains to clarify whether the translocation of GK following its dissociation from GKRP and/or increased free GK in the cytoplasm is necessary for increasing glucose phosphorylation in the liver.

GKRP seems to play a critical role in importing GK into the nucleus and in localizing GK in the nuclear compartment. Mice mutant for GKRP exhibit cytoplasmic localization of GK even at low blood glucose levels (11, 16). When cultured HeLa cells (9, 36) and human embryonic kidney cells (6) are transfected with the GK gene alone, cytoplasmic GK does not accumulate in the nucleus even in the presence of Leptomycin B (36), which inhibits signal-mediated nuclear export (14, 21, 22). When GK is coexpressed with GKRP in these cells, GK accumulates in the nucleus with GKRP (6, 36). These findings suggest that the presence of GKRP is essential for importing GK into the nucleus. Furthermore, it has been shown that a mutant form of GK with reduced affinity for GKRP does not concentrate in the nucleus even in the presence of GKRP at low glucose levels (9, 36), suggesting that GKRP binding is essential for importing GK into the nucleus. So far, there are few studies that explore the relationship between the switch in net hepatic glucose balance from uptake to production and the fate of GK translocated into the cytoplasm. Brown et al. (7) have shown that GK returns to the nucleus from the cytoplasm within 30 min after reduction of glucose concentration from 20 to 5 mM in cultured hepatocytes. We (38) have shown that in a conscious dog, increased rates of net hepatic glucose uptake caused by fructose return to the basal rate within 30 min of terminating the infusion of the sugar. It is possible, therefore, that the switch in net hepatic glucose balance from uptake to production relates to the nuclear returning of GK.

Shiotaka et al. (36) have shown the presence of a functional nuclear export signal sequence in the GK molecule. If the binding of GK with GKRP is essential for the accumulation of GK in the nucleus as mentioned above, GK may dissociate from GKRP in the nucleus before its export to the cytoplasm. Agius and Stubbs (3) have demonstrated that a glucose analog, which is not phosphorylated by GK, mimics the effect of glucose to stimulate GK translocation, suggesting that this effect could be mediated by the rise in intracellular concentra-
tion of the sugar itself, not by a metabolite of glucose or by GK-catalyzed phosphorylation. The intracellular concentration of glucose in hepatocytes is usually equal to or slightly higher than the plasma concentration (48) and rapidly equilibrates with plasma glucose when the plasma glucose level is changed (31). The molecular mass of glucose, below the exclusion limit of the nuclear pore (32), should allow passive diffusion through the nuclear membrane. So far, however, the mechanisms by which the rise in plasma glucose and insulin mediate the dissociation of GK from GKR P in the nucleus and/or translocation of GK from the nucleus to the cytoplasm remain unknown.

In the present study, the translocation or the redistribution of GKR P was not detected in response to glucose feeding, hyperglycemia, and/or hyperinsulinemia. In agreement with our results, studies on hepatocytes using immunohistochemical (7, 20) or digitonin-permeabilization techniques (1, 2) showed that GKRP does not translocate during incubation with fructose or a high concentration of glucose. On the other hand, Toyoda et al. (43) reported that in rat livers perfused with 5 mM glucose, GK locates in the nucleus, but perfusion with 20 mM glucose leads to the partial translocation of GKRP to the cytoplasm. However, in the same report (43), GKRP was exclusively found in the nuclear fraction of normal-fed rat livers. With the use of confocal microscopy and quantitative imaging, Muhktar et al. (30) demonstrated that in contrast to their previous report (2), the GKR P also translocates from the nucleus to the cytoplasm in response to glucose or sorbitol. Recently, Shiota et al. (36) have shown that GKR P is distributed in both the cytoplasm and the nucleus in the absence of GK in culture HeLa cells transfected with a GKR P gene and that the coexpression of GK with GKR P caused preferential localization of GKR P in the nucleus. Taken together with the evidence that the binding of GK with GKPR is essential for importing GK into the nucleus, they propose that GKR P shuttles GK back to the nucleus when GK translocation is stimulated (36). Because increases in such shuttling of GKR P may not necessarily be accompanied by altered distribution of this protein between the nucleus and the cytoplasm, our study might not have detected the small change.

It should be noted that GK and GKR P are not expressed homogeneously in all the parenchymal cells in the liver. GK is expressed from periportal to perivenous areas in an increasing gradient (10, 28), and even in perivenous areas, the intensity of immunofluorescence of GK and GKR P varies among hepatocytes (data not shown). Furthermore, the extent of GK translocation in response to increased plasma glucose may differ throughout the liver lobule. To avoid intentional selection and to obtain results reflecting changes in the whole liver, we randomly selected five microscopic areas in the section from each animal. To identify GK-positive cells, we selected cells with a high immunofluorescence intensity of GKRP in the nucleus compared with controls stained with preimmune serum, because GK is always coexpressed with GKRP. We did not detect any cells in which GK was present in the nucleus in the absence of GKRP (data not shown). As a result, cells might not have been selected in which GKRP was originally located in the cytoplasm or GKRP was largely exported from the nucleus in response to the stimulus. It is possible, therefore, that the ratio of nuclear to cytoplasmic GKRP described in this study overestimates its nuclear localization.

In summary, the present study demonstrates in conscious rats that GK quickly translocates to the cytoplasm, which may explain the switch in net hepatic glucose balance from output to uptake in response to glucose ingestion. Also, the rise in plasma glucose and insulin in the physiological range, major mediators of the switching of net hepatic glucose balance, may cause GK translocation. These data support the hypothesis that the translocation of GK from the nucleus to the cytoplasm following the dissociation of this enzyme from GKR P plays a physiological role in switching net hepatic glucose balance from production to uptake after a meal. Insulin has been reported to inhibit glucose-6-phosphatase activity in the short term by its direct action on the liver (15) probably via the lipid products of phosphatidylinositol 3-kinase activity (25). It is likely, therefore, that insulin may rapidly switch hepatic glucose balance to uptake by acting simultaneously to activate GK and inhibit glucose-6-phosphatase. On the other hand, glucagon has been reported to activate glucose-6-phosphatase in the short term (18) and to inhibit GK translocation in cultured hepatocytes (1, 7, 8) by decreasing the intracellular concentration of fructose-1-phosphate (8). It is possible, therefore, that a part of the regulation of hepatic glucose flux by insulin and glucagon takes place at the site of GK/glucose-6-phosphatase.

REFERENCES


