

# Hyperglycemia Contributes to Impaired Insulin Response in GK Rat Islets

Zong-Chao Ling, Cao Hong-Lie, Claes-Göran Östenson, Suad Efendic, and Akhtar Khan

**Insulin secretion and glucose metabolism were compared in pancreatic islets from type 2 diabetic GK rats treated with phlorizin or vehicle. Treatment of control and GK rats with phlorizin for 30 days did not affect body weight, islet glucose utilization, or islet glucose oxidation. In phlorizin-treated GK rats, glucose-induced insulin release was about twofold higher at 11.0 and 16.7 mmol/l glucose compared with vehicle-treated GK rats, whereas phlorizin had no effect on control Wistar rats. However, also in phlorizin-treated GK rats, the amount of insulin released by the islets was significantly less than that from control rats ( $5.29 \pm 0.33$  vs.  $7.50 \pm 1.31$  pmol  $\cdot$  min<sup>-1</sup>  $\cdot$  islet<sup>-1</sup> at 16.7 mmol/l glucose;  $P < 0.001$ ). Islet glucose-6-phosphatase activity was significantly higher in GK rats than in control rats; phlorizin treatment significantly decreased this activity. These findings demonstrate that hyperglycemia per se constitutes an important factor for impaired insulin release in GK rats. Correction of hyperglycemia normalizes islet glucose-6-phosphatase activity, which may be an underlying factor for the partial improvement of glucose-induced insulin release. *Diabetes* 50 (Suppl. 1):S108-S112, 2001**

**T**ype 2 diabetes develops because of impaired insulin release and/or decreased insulin sensitivity (1,2). The spontaneously diabetic Goto-Kakizaki (GK) rat is a nonobese animal model of type 2 diabetes characterized by a deficient insulin response to glucose in vivo and in vitro (3-6). The mechanism of the impaired insulin release in the GK rat is not clear; however, a number of abnormalities in islet glucose metabolism have been demonstrated. Thus, glucose utilization and oxidation were markedly increased in GK rat islets (6,7). In addition, islet glucose cycling (GC) was significantly higher in GK rats compared with control Wistar rats. GC is a futile cycle in which glucose is phosphorylated to glucose-6-phosphate (G6P) by glucokinase and then dephosphorylated to glucose by glucose-6-phosphatase (G6Pase) with the resulting consumption of one molecule of ATP (8). Increased GC may decrease the cyto-

plasmic ATP/ADP ratio, which leads to incomplete closure of ATP-sensitive K<sup>+</sup> channels, deficient membrane depolarization, less increase in cytoplasmic calcium, and impaired insulin release (9). In this context, it is of interest that hepatic GC is increased in patients with mild hyperglycemia with nearly normal hepatic glucose production (10), indicating that increased G6Pase is an early feature of glucose intolerance.

Evidence has been presented that hyperglycemia per se is involved in the impairment of insulin secretion in type 2 diabetes (11-14). It was also shown that in 90% pancreatized rats, correction of hyperglycemia by phlorizin (Phl) improves insulin secretion (15). In experiments with neonatally streptozotocin-induced diabetic rats, treatment with insulin normalized blood glucose levels and partially improved insulin release (16). Furthermore, insulin treatment improved insulin response to glucose in patients with type 2 diabetes (17). In our previous studies with glucose-intolerant F<sub>1</sub>-hybrids of GK and Wistar rats, significantly impaired islet insulin secretion was not corrected after transplantation to immune-tolerant NOD mice (18). However, this does not exclude the fact that hyperglycemia contributes to a severe defect in insulin responsiveness in GK rats. Therefore, the present study was designed to evaluate insulin response in islets of GK rats after long-term improvement of glycemia by Phl treatment.

## RESEARCH DESIGN AND METHODS

**Chemicals.** [<sup>14</sup>C]Glucose (251.0 mCi/mmol) and [<sup>3</sup>H]glucose (15.7 Ci/mmol), reported to be radiochemically >98% pure, were purchased from Dupont-NEN (Boston, MA). By high-performance liquid chromatography on an Aminex HPX-87P column (Bio-Rad, Richmond, CA) with water as a solvent at 85°C, the mixture of [<sup>14</sup>C]- and [<sup>3</sup>H]glucose gave two peaks: one with the mobility of glucose and the other with <1% as much of <sup>3</sup>H and <sup>14</sup>C. The glucose peak was collected and aliquotted into vials and freeze-dried for use in the incubations. [1-<sup>14</sup>C]G6P (disodium salt) (57.7 mCi/mmol) was obtained from Dupont-NEN. Glucose and Amberlite MB-3 mono-bed resin was purchased from BDH (Pool, U.K.). Collagenase A was from Boehringer Mannheim (Mannheim, Germany), and RPMI 1640 was from Statens Veterinärmedicinska Anstalt (Uppsala, Sweden). DABA (3,5,6-diaminobenzoic acid dihydrochloride) was obtained from Aldrich (Steinheim, Germany). HEPES (*N*-[2-hydroxyethyl] piperazine-*N*-[2-ethanesulfonic acid]), G6P, mannose-6-phosphate, fetal calf serum, B-glycerol-3-phosphate (gly-3-P), propylene glycol, phlorizin (phloretin-2-*B*-*D*-glucoside), Triton X-100, and bovine serum albumin (fraction V) were purchased from Sigma (St. Louis, MO).

**Animals.** Male GK rats, 2-3 months of age, were from our colony. Age-matched male Wistar control rats were purchased from B & K Universal (Sollentuna, Sweden). All rats were fed ad libitum with a pelleted lab diet (B & K Universal) and water. They were killed by decapitation, and pancreases were removed for islet isolation. Blood was collected at decapitation. Three groups of animals were used for the study. In the first series of experiments, untreated Wistar control ( $n = 8$ ) and GK ( $n = 8$ ) rats were used. In the second, 16 GK rats were fasted overnight, and the morning glucose concentration was measured in the tail vein blood. Then a glucose tolerance test was performed. An aqueous solution (250  $\mu$ l; 2 g glucose/kg body wt) was injected intraperitoneally. Glucose concentration was determined in blood collected from the tail vein before and 30 and 120 min after injection. The rats then had free access to food and water and were divided into two groups (eight animals each). One group was injected with Phl in propylene glycol and the other with propylene glycol (vehicle) alone. Phl

From the Department of Molecular Medicine, Endocrine and Diabetes Unit, Rolf Lufts Centrum for Diabetes Research, Karolinska Hospital, Karolinska Institute, Stockholm, Sweden.

Address correspondence and reprint requests to Akhtar Khan, Department of Molecular Medicine, Endocrine and Diabetes Unit, Karolinska Hospital, S-171 76 Stockholm, Sweden. E-mail: akhtar.khan@molmed.ki.se.

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ER, endoplasmic reticulum; G6Pase, glucose-6-phosphatase; GC, glucose cycling; G6P, glucose-6-phosphate; gly-3-P, B-glycerol-3-phosphate; KRB, Krebs-bicarbonate buffer; Phl, phlorizin.

TABLE 1  
Body weight and blood glucose concentrations in fed control Wistar and GK rats treated with Phl or vehicle

	Control rats		GK rats	
	Phl-treated	Vehicle	Phl-treated	Vehicle
<i>n</i>	8	8	5	5
Body weight (g)				
Before treatment	220 ± 12	221 ± 13	172 ± 13	174 ± 14
After treatment	281 ± 13	278 ± 17	225 ± 19	246 ± 14
Blood glucose at decapitation (mmol/l)	5.6 ± 0.2	5.2 ± 0.3	7.7 ± 0.3	
15.1 ± 1.2*				

Data are means ± SE. \**P* < 0.001, Phl treated vs. vehicle.

(0.4 g/kg body wt), made up as a 20% solution in vehicle, or vehicle alone was administered by subcutaneous injection in the morning (8:00 A.M.) and evening (8:00 P.M.) daily for 30 days. After 20 days of treatment, a second glucose tolerance test was performed, and the rats were killed after 30 days of treatment. In the third series of experiments, 10 Wistar rats were divided into two groups (five animals each); one group was injected with Phl and the other with the vehicle as described above for 30 days. After 20 days of treatment, a glucose tolerance test was performed, and the rats were killed after 30 days of treatment. **Experiments with isolated islets.** Islets were isolated as previously described using collagenase digestion (7). Islets from control and GK rats were used to measure glucose utilization, glucose oxidation, DNA content, G6Pase activity, and insulin release. For glucose utilization and glucose oxidation measurements, four batches of 25 islets were placed in an incubation vial with either 5.5 or 16.7 mmol/l glucose (in duplicate), 0.5% bovine serum albumin (fraction V), and 1 μCi [5-<sup>3</sup>H]glucose and 1 μCi [U-<sup>14</sup>C]glucose in 100 μl Krebs-bicarbonate buffer (KRB), pH 7.4. Each vial with its content was placed in a scintillation bottle, sealed, and gassed with O<sub>2</sub>/CO<sub>2</sub> (19:1 by volume) for 3 min. After a 90-min incubation at 37°C, 100 μl 10% perchloric acid was injected into the vial, and 250 μl Hyamine (Packard, Meriden, CT) and 250 μl water were injected into the bottle to absorb <sup>14</sup>CO<sub>2</sub> and <sup>3</sup>H<sub>2</sub>O, respectively. Parallel incubations were performed without islets. <sup>14</sup>CO<sub>2</sub> and <sup>3</sup>H<sub>2</sub>O were collected overnight. The vial with its acidified content was removed, and 10 ml scintillation fluid (Ultima Gold; Packard) was added to the bottle, which was then assayed for the radioactivity in a liquid scintillation spectrophotometer (Tri-Carb 1900 TR liquid scintillation analyzer; Packard).

To measure G6Pase activity, batches of 50 islets (in duplicate) were put in tubes containing 50 mmol/l HEPES buffer (pH 7.4), 20 mmol/l gly-3-P, 4 mmol/l [1-<sup>14</sup>C]G6P (0.5 μCi) in a volume of 0.2 ml. Gly-3-P was added to inhibit nonspecific phosphatase activity (19). The tubes were immersed in ethanol and dry ice for 20 s to permeabilize the islets. Other batches of 50 islets (in duplicate) were sonicated for 5–10 s in 50 mmol/l HEPES (pH 7.4), and then identical concentrations of gly-3-P and G6P were added into the tube. The tubes were then incubated at 37°C in a water bath with gentle shaking for 20 min. Parallel incubations were performed without islets. The reaction was stopped by placing the tubes in ice water with the addition of 0.3 ml of 0.3 mol/l ZnSO<sub>4</sub> and after mixing 0.3 ml of saturated solution of Ba(OH)<sub>2</sub>. Then 2 mg of glucose was added to each tube, mixed, and centrifuged in an Eppendorf centrifuge (10,000g) for 2 min. The supernatant was passed through an MB-3 monobed ion-exchange resin column, and the effluent was evaporated to dryness. The residue was dissolved in 1 ml water, and 500 μl was counted for radioactivity. The glucose concentration in the remainder was measured with glucose oxidase (Glucose Analyzer, Model 23A; Yellow Springs Instruments, Yellow Springs, OH).

For insulin secretion studies, islets were preincubated for 30 min at 3.3 mmol/l glucose in KRB containing 2 mg/ml bovine serum albumin. Batches of three islets were then incubated at 37°C for 1 h in 300 μl KRB containing different glucose concentrations (3.3, 8.3, 11.0, and 16.7 mmol/l). After incubation, an aliquot of the medium was stored at -70°C for insulin assay (20). Islet DNA content in a batch of 25 islets was assayed using a fluorometric method modified by Hinegardner (21).

**Calculations and statistical analysis.** The <sup>14</sup>CO<sub>2</sub> and <sup>3</sup>H<sub>2</sub>O formed by the islets were taken as the difference in <sup>14</sup>C in CO<sub>2</sub> and <sup>3</sup>H in H<sub>2</sub>O incubated in the presence and absence of islets. The amounts of <sup>14</sup>CO<sub>2</sub> and <sup>3</sup>H<sub>2</sub>O formed (picomoles of glucose equivalent) were calculated by dividing the radioactivity (dpm) in the CO<sub>2</sub> and H<sub>2</sub>O by the specific activity (dpm per picomole) of [U-<sup>14</sup>C]glucose and [5-<sup>3</sup>H]glucose in the medium. The yield of <sup>3</sup>H<sub>2</sub>O was corrected for the recovery of <sup>3</sup>H by incubating <sup>3</sup>H<sub>2</sub>O under identical conditions. The specific activity of [1-<sup>14</sup>C]G6P was calculated from the quantity of G6P in the incubate (picomoles) and the amount of <sup>14</sup>C in that G6P. The dpm in the 2 mg glu-

cose added as a carrier after stopping the incubation were calculated from the <sup>14</sup>C activity (dpm) in the effluent from the ion-exchange column and the amount of glucose in the effluent (usually ~1.5 mg). The amount of G6P hydrolyzed to glucose was then calculated by dividing the dpm in the 2 mg glucose by the specific activity of [1-<sup>14</sup>C]G6P in dpm per picomole after subtracting the dpm in the 2 mg glucose when an identical incubation was done, except for the absence of islets or microsomes.

Results are expressed as means ± SE. Significance of difference was assayed by a Student's *t* test for unpaired observations.

## RESULTS

**Characteristics of animals.** Control Wistar (*n* = 18) and GK (*n* = 24) rats had similar body weight (229 ± 11 vs. 204 ± 15 g), whereas nonfasting blood glucose concentrations were significantly higher in GK rats than in control rats at the start of the experiment (12.7 ± 0.7 vs. 6.4 ± 0.9 mmol/l; *P* < 0.001). Islet DNA content was similar in control and GK rats (25.6 ± 1.2 vs. 27.0 ± 0.8 ng/islet). Phl had no effect on body weight in either GK or control rats (Table 1). Phl significantly decreased blood glucose in fed GK rats but did not normalize it (Table 1). The compound had no effect on glycemia in control Wistar rats. In GK rats, Phl had only a modest effect on early (30-min) glycemic response after intraperitoneal administration of glucose (Table 2). However, at 120 min, blood glucose levels were comparable in Phl-treated GK and control animals (Table 2).

**Insulin release.** In control islets, glucose stimulated insulin release in a dose-dependent manner (Fig. 1). At 16.7 mmol/l glucose, insulin release was 5.7 times higher than that at 3.3 mmol/l glucose. In islets from GK rats, glucose-induced insulin release was significantly impaired at all concentrations and was only 1.6 times higher at 16.7 mmol/l glucose than at 3.3 mmol/l glucose (Fig. 2). Long-term treatment of control and GK rats with vehicle did not affect the insulin responses (Figs. 1 and 2). In islets from Phl-treated control rats, glucose-induced insulin release was similar to that in vehicle-treated animals at all glucose concentrations (Fig. 1). In contrast, in Phl-treated GK rats, glucose-induced insulin release was about twofold higher at 11 and 16.7 mmol/l glucose (Fig. 2). However, also in Phl-treated animals, the amount of insulin released by GK rat islets was significantly lower than that from control rats at all stimulatory glucose concentrations (e.g., 53.0 ± 3.3 vs. 75.2 ± 13.1 μU · islet<sup>-1</sup> · h<sup>-1</sup> at 16.7 mmol/l glucose; *P* < 0.01).

**G6Pase activity.** G6Pase activity measured at 4 mmol/l G6P was significantly higher in both permeabilized and sonicated islets from GK rats than those from control Wistar rats (Table 3). Islet G6Pase activity was lower in Phl-treated rats than in vehicle-treated GK rats, whether permeabilized or sonicated islets were used, and was comparable to that in Wistar rat islets.

TABLE 2

Glucose tolerance test with intraperitoneal injection of glucose (2 g/kg body wt) in overnight fasted Wistar (control) and GK rats before and after 21 days of treatment with Phl or vehicle

	Blood glucose concentration (mmol/l)					
	Control ( <i>n</i> = 5 + 5)			GK ( <i>n</i> = 8 + 8)		
	0 min	30 min	120 min	0 min	30 min	120 min
Before Phl treatment	—	—	—	3.8 ± 0.3	14.6 ± 1.3	10.4 ± 0.9
After Phl treatment	3.6 ± 0.2	8.7 ± 0.6	3.9 ± 0.1	3.4 ± 0.3	16.1 ± 1.5	4.3 ± 0.6
Before vehicle	—	—	—	3.5 ± 0.3	15.2 ± 1.5	9.6 ± 0.8
After vehicle	3.6 ± 0.4	7.9 ± 0.7	3.9 ± 0.4	5.9 ± 0.8	21.0 ± 1.1	11.3 ± 0.9*

Data are means ± SE. \**P* < 0.001, Phl-treated vs. vehicle rats at 120 min.

**Glucose utilization and oxidation.** Islets from Phl-treated GK rats at 5.5 and 16.7 mmol/l glucose used and oxidized similar amounts of glucose as islets from vehicle-treated GK rats. Glucose utilization and oxidation were significantly higher at 16.7 mmol/l than at 5.5 mmol/l glucose in both Phl- and vehicle-treated animals (*P* < 0.001; Table 4).

## DISCUSSION

Previously, we have reported that in islets from GK rats, glucose-induced insulin release is impaired although these islets use and oxidize two to three times more glucose than islets from control rats (7). The rate of GC was significantly higher in GK islets, which we interpreted as an indication of increased activity of islet G6Pase (6). The present study demonstrates that islet G6Pase activity is indeed increased in this animal model of type 2 diabetes. In addition, we present evidence that improved glycemic control by Phl normalizes islet G6Pase activity and partially improves glucose-induced insulin release without affecting the rate of glucose utilization and oxidation. Phl had only a modest effect on early glycemic response to intraperitoneal glucose administration. This result implies that also after food ingestion, short-term hyperglycemia may occur in Phl-treated GK rats. Hence, our experimental con-

ditions may underestimate the significance of hyperglycemia for defective insulin response in GK rats.

Chronic hyperglycemia is known to impair insulin secretion in response to glucose. In the hyperglycemic model of partial pancreatectomized rats (11,12,15) and in rats infused with high glucose (22) as well as in studies with rat and human islets cultured at high glucose concentrations (23,24), glucose-induced insulin release was impaired. Conversely, partial or even total restoration of insulin response was achieved by normalizing the glycemia by treating the animals with Phl (15) or insulin (16).

To evaluate the mechanisms behind impaired glucose-induced insulin release and increased G6Pase activity in GK rat islets, we treated the animals with Phl for 30 days to achieve normoglycemia and then used their islets for the study. The improved insulin response by Phl treatment suggests that hyperglycemia, at least partially, contributes to impaired insulin responsiveness in GK rat islets. However, in our previous studies with GK-Wistar hybrid rats with only mild glucose intolerance, impaired insulin response was not related to hyperglycemia (18). Thus, the maintenance for 4 weeks of transplanted hybrid rat islets under the kidney capsule of normoglycemic nude mice did not improve glucose-induced

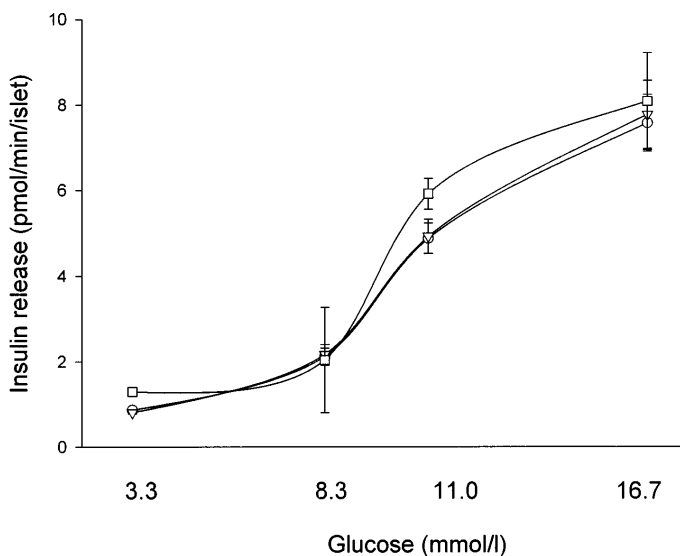


FIG. 1. Glucose-induced insulin release in islets from fed control Wistar rats (○, untreated; ▽, vehicle-treated; □, Phl-treated). Data are means ± SE of five rats in each group.

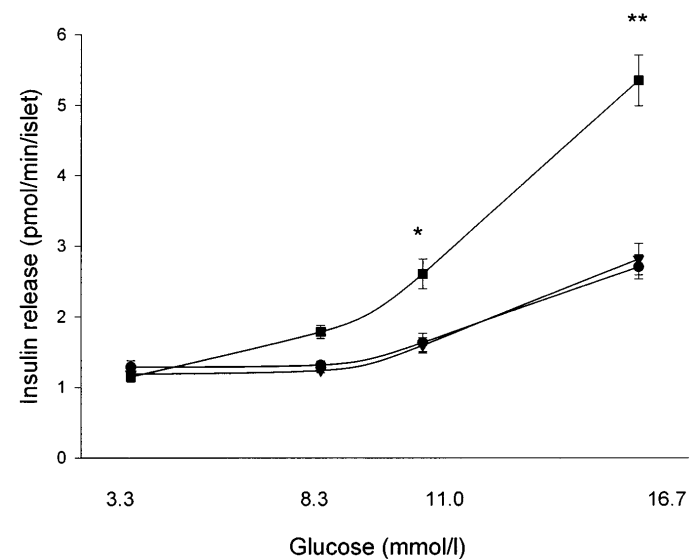


FIG. 2. Glucose-induced insulin release in islets from GK rats (●, untreated; ▽, vehicle-treated; ■, Phl-treated). Data are means ± SE of five rats in each group. \**P* < 0.05; \*\**P* < 0.01.

TABLE 3

Islet G6Pase activity (picomoles per nanograms of islet DNA per hour) in isolated islets from untreated control Wistar (control) and GK rats and from GK rats treated with Phl or vehicle

Rats	n	Permeabilized	Sonicated
Control	5	1.82 ± 0.12	3.74 ± 0.70*
GK	5	4.51 ± 0.14†	7.75 ± 0.89*†
GK (Phl treated)	5	2.59 ± 0.43	4.27 ± 0.62‡
GK (vehicle-treated)	5	4.96 ± 0.64§	6.61 ± 0.47‡§

Data are means ± SE. \* $P < 0.05$  vs. permeabilized islets; † $P < 0.01$  vs. control in permeabilized and sonicated islets; ‡ $P < 0.05$  vs. permeabilized islets; § $P < 0.01$  vs. Phl treated in permeabilized and sonicated islets.

insulin response. These results support the important role of heredity for the impaired insulin secretion in GK rats.

G6Pase is mainly present in the liver and kidney. The enzyme is also present in the pancreatic islets of mammals (25–27). G6Pase consists of at least two components: a G6P-specific transporter through the endoplasmic reticulum (ER) and a relatively nonspecific phosphohydrolase situated on the luminal surface of the ER (28). The activity of G6Pase in intact ER membrane estimates the translocase function, whereas the enzyme activity after complete disruption of the membrane provides a measure of the hydrolase component. Detergent plus sonication is usually used to completely disrupt the membrane (29). In permeabilized cells, the ER membrane remains intact (30). Hence, we have used permeabilized and sonicated islets to measure the translocase and hydrolase components of the enzyme, respectively. Islets were sonicated without detergents because we did not find any difference in enzyme activity, whether the islets were sonicated with or without detergent (31). In the present study, improvement of insulin response appeared in parallel with the normalization of islet G6Pase activity. An important question, then, is whether the normalization of islet G6Pase activity is one of the factors behind the improved insulin response in Phl-treated GK rats. We have previously showed parallel inhibition of glucose-induced insulin release and increased islet G6Pase activity in dexamethasone-treated *ob/ob* mice (32) without affecting glucose utilization and oxidation. Also, in transgenic mice with overexpressed glucocorticoid receptors in the  $\beta$ -cells, decreased glucose-induced insulin release was paralleled by increased islet G6Pase activity with normal glucose utilization and oxidation (33). More direct evidence for the involvement of

TABLE 4

Glucose utilization, glucose oxidation, and DNA content in islets from GK rats treated with Phl or vehicle

	Glucose (mmol)	Vehicle	Phl treated
Glucose utilization (pmol · ng <sup>-1</sup> DNA · h <sup>-1</sup> )	5.5	2.31 ± 0.27	1.83 ± 0.20
Glucose oxidation (pmol · ng <sup>-1</sup> DNA · h <sup>-1</sup> )	16.7	4.83 ± 1.03	3.97 ± 0.66
DNA (ng/islet)	5.5	0.48 ± 0.05	0.44 ± 0.88
	16.7	1.32 ± 0.18	1.32 ± 0.26
	—	26.1 ± 6.1	34.9 ± 5.6

Data are means ± SE.

G6Pase in insulin release was provided recently by the demonstration that overexpression of the catalytic subunit of the hepatic G6Pase complex in an insulinoma cell line resulted in increased GC and decreased insulin release (34); nearly 10-fold increased G6Pase activity was accompanied by a 30% reduction in insulin secretion and a similar reduction in glucose utilization. In the present study, glucose utilization in GK rat islets did not change in proportion to decreased islet G6Pase activity and improved insulin release after Phl treatment. The reason for this discrepancy is not clear but may be due to a markedly different regulation of glucose metabolism in tumor cells compared with primary cells.

Because improvement of glycemia in GK rats had no effect on islet glucose utilization and oxidation, we conclude that neither glycolytic nor mitochondrial functions were modified by Phl. In GK islets, two to three times more glucose is used and oxidized than in control islets; therefore, proportionately more ATP would be generated, far exceeding the amount consumed by the increased GC (7). However, because the cycling is a cytoplasmic process, it may be speculated that it could decrease the ATP/ADP ratio in a critical region of the cell (at the ATP-regulated K<sup>+</sup> channels) and consequently affect insulin release.

In conclusion, in GK rats, improved glycemic control by Phl enhanced but did not normalize glucose-induced insulin release. In addition, Phl treatment normalized the augmented activity of islet G6Pase. Increased G6Pase activity due to hyperglycemia may participate in the impairment of insulin release (glucotoxicity).

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