Regulation of Insulin Secretion in Relation to Nitric Oxide, Carbon Monoxide and Acid alpha-Glucoside Hydrolase Activities

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Dysfunction of the Islet Lysosomal System Conveys Impairment of Glucose-Induced Insulin Release in the Diabetic GK Rat*

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ABSTRACT

Accumulated evidence links an important signal involved in glucose-stimulated insulin release to the activation of the islet lysosomal glycogenolytic enzyme acid glucan-1,4-α-glucosidase. We have analyzed the function of the lysosomal system/lysosomal enzyme activities in pancreatic islets of young (6–8 weeks), spontaneously diabetic, GK (Goto-Kakizaki) rats and Wistar control rats in relation to glucose-induced insulin release. The insulin secretory response to glucose was markedly impaired in the GK rat, but was restored by the adenylate cyclase activator forskolin. Islet activities of classical lysosomal enzymes, *e.g.*. acid phosphatase, *N*-acetyl-β-D-glucosaminidase, β-glucuronidase, and cathepsin D, were reduced by 20–35% in the GK rat compared with those in Wistar controls. In contrast, the activities of the lysosomal α-glucosidehydrolases, *i.e.*. acid glucan-1,4-α-glucosidase and acid α-glucosidase, were increased by 40–50%. Neutral α-glucosidase (endoplasmic reticulum) was unaffected. Comparative analysis of liver tissue showed that lysosomal enzyme activities were of the same magnitude in GK and Wistar rats. Notably, in Wistar rats, the activities of acid glucan-1,4-α-glucosidase and acid α-glucosidase were approximately 15-fold higher in islets than in liver. Other lysosomal enzymes did not display such a difference. Normalization of glycemia in GK rats by phlorizin administered for 9 days did not influence either the lysosomal α-glucosidehydrolase activities or other lysosomal enzyme activities in GK islets. Finally, the pseudotetrasaccharide acarbose, which accumulates in the lysosomal system, inhibited acid glucan-1,4-α-glucosidase activity in parallel with its inhibitory action on glucose-induced insulin release in intact Wistar islets, whereas no effect was recorded for either parameter in intact GK islets. In contrast, acarbose inhibited the enzyme activity equally in islet homogenates from both GK and Wistar rats, showing that the catalytic activity of the enzyme itself in disrupted cells was unaffected. We propose that dysfunction of the islet lysosomal/vacuolar system is an important defect impairing the transduction mechanisms for glucose-induced insulin release in the GK rat. (*Endocrinology* **140:** 3045–3053, 1999)

THE SECRETION of insulin is governed by a complex chain of events occurring in response to different metabolic, nervous, and hormonal factors. Insulin release stimulated by glucose, the major insulin secretagogue, involves the closure of ATP-sensitive K^+ channels, with subsequent depolarization and opening of the voltage-dependent Ca^{2+} channels. This leads to an increase in intracellular Ca^{2+} and subsequent exocytosis of insulin. The latter may be further modulated by activation of different second messengers, such as various metabolites of inositol phospholipids, cAMP, arachidonic acid, and others (1, 2). We have previously proposed that one of these multiple signals in glucose-stimulated insulin release is transduced through the vacuolar system involving the activation of a lysosomal acid glucan-1,4-α-glucosidase (3–11), a member of the α-glucosidehydrolase family of enzymes. This lysosomal glucose-producing enzyme preferentially cleaves α-1,4-linked glucose polymers and might thus attack certain pools of islet vacuolar glycogen to produce high local concentrations of free glucose, which, in turn, could act as a transducer (cybernetic, metabolic, or osmotic) in the multifactorial process of insulin secretion. Hence, in accordance with this idea, a close relationship between islet acid glucan-1,4-α-glucosidase activity and insulin release was recently observed in a series of experiments with isolated islets, where three different and selective α-glucosidehydrolase inhibitors, *i.e.* emiglitate, miglitol, and acarbose, were found to suppress glucose-stimulated insulin release in parallel with their inhibitory effect on islet glucan-1,4-α-glucosidase activity (6–12). Moreover, previous *in vivo* experiments (3, 4, 13, 14) showed a positive correlation between islet glucan-1,4-α-glucosidase activity and glucose-stimulated insulin secretion both in normal mice and in the insulin-hypersecreting *ob/ob* mouse, an animal model of the obese type of noninsulin-dependent diabetes mellitus (NIDDM), whose islets display a good insulin response to glucose. The question then arose whether other types of spontaneous NIDDM in animals, *e.g.* with a reduced insulin response to glucose, would disclose any impairment in the link between lysosomes/lysosomal enzyme activities and glucose-induced insulin release.

The aim of the present investigation was therefore to study whether a nonobese animal model of NIDDM, the Goto-Kakizaki (GK) rat, which in contrast to the *ob/ob* mouse is known to have a very poor insulin response to glucose (15–

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18), would display any putative dysfunction in the relation between the islet lysosomal system/lysosomal acid α-glucosidehydrolase activities and insulin storage and secretion. Additionally, we performed comparative analyses of lysosomal enzyme activities in liver tissue. The GK rat is known as a nonobese mildly diabetic rat that was developed by inbreeding Wistar rats with the highest blood glucose levels during an oral glucose tolerance test (15, 16), and recent studies have shown that their diabetic state may be mainly explained by a defect in glucose-induced insulin secretion, which was markedly impaired in both perfused pancreas and isolated islets of these rats (17, 18). In the present study we used one fuel secretagogue, glucose, and the adenylate cyclase activator forskolin, as the interaction between glucose, on the one hand, and the cAMP system, on the other, is known to play a pivotal role in the appropriate release of insulin (2). Moreover, data from our previous experiments have suggested that glucose-induced insulin release, but not cAMP-activated insulin release, is dependent on islet acid glucan-1,4-α-glucosidase activity and normal function of the lysosomal/vacuolar system (4, 6, 7, 9, 13, 19). We also studied islet lysosomal function in GK rats treated for 9 days with phlorizin to test whether normalization of their blood glucose levels would affect the lysosomal system. Finally, we investigated in both GK and Wistar rats the ability of the pseudotetrasaccharide acarbose, a potent inhibitor of islet acid glucan-1,4-α-glucosidase (9), to enter into the lysosomal system and modulate enzyme activity as well as glucose-induced insulin secretion.

Materials and Methods

Animals

Age- and sex-matched GK rats of the Stockholm colony and Wistar controls (B&K Universal, Sollentuna, Sweden) were used. They were fed a standard pellet diet (B&K Universal) and tap water *ad libitum* before and during the experiments.

Drugs and chemicals

Collagenase (CLS 4) was obtained from Worthington Biochemical Corp. (Freehold, NJ). Forskolin, phlorizin, and methylumbelliferyl-coupled substrates were purchased from Sigma Chemical Co. (St. Louis, MO). BSA was obtained from ICN Biomedicals, Inc. (High Wycombe, UK). The pseudotetrasaccharide acarbose was supplied by Bayer Corp. (Leverkusen, Germany). All other chemicals were purchased from Merck & Co., Inc. (Darmstadt, Germany). The RIA kits for insulin determination were obtained from Diagnostika (Falkenberg, Sweden).

In vivo studies

Young adult GK and Wistar rats, 1–2 months of age, were injected iv with glucose (11.1 mmol/kg), and serial blood sampling by the retrobulbar approach was performed as previously described (20). The volume load was 5 μl/g rat. Concentrations of insulin and glucose in plasma were determined by the methods of Heding (21) and Bruss and Black (22), respectively. To elucidate whether normalization of blood glucose levels in the GK rat would affect islet lysosomal enzyme activities, one group of GK rats was injected with phlorizin, and another group was injected with solvent (propylene glycol). Phlorizin (0.4 g/kg BW·day), made up as a 20% solution in propylene glycol, or propylene glycol alone was administered as a sc injection every morning and afternoon for 9 days. Wistar control rats receiving solvent were included. Islets were then isolated as described below.

Islet studies

Young adult GK and Wistar rats were killed by decapitation and immediately subjected to a retrograde injection of a collagenase solution via the bile-pancreatic duct for preparation of isolated pancreatic islets (23). The freshly isolated islets were either collected for lysosomal enzyme analysis or used for insulin secretion studies. For determination of lysosomal enzyme activities, 150–200 islets were thoroughly washed in glucose-free Hanks' solution and collected and stored in 300–400 μl acetate-EDTA buffer (1.1 mmol/liter EDTA and 5 mmol/liter acetate, pH 5.0) at −20 C. After thawing in an ice bath and subsequent sonification, the islet homogenates were analyzed for enzyme activities. Cathepsin D (pH 4.0) was assayed with a slightly modified scaled down version of the method of Barrett (24). The procedure for determination of acid phosphatase (pH 4.5), acid α-glucosidase (pH 4.0), β-glucuronidase (pH 4.0), *N*-acetyl-β-D-glucosaminidase (pH 5.0), and neutral α-glucosidase (pH 6.5) (endoplasmic reticulum) with methylumbelliferyl-coupled substrates has previously been described (3). Glucan-1,4-α-glucosidase activity with glycogen as substrate was determined at pH 4.0 as previously described in detail (5, 25). Protein was analyzed according to the method of Lowry *et al.*. (26). Extraction and assay of islet insulin content were performed with acid-ethanol as previously described (21, 27).

For insulin secretion studies, freshly isolated islets were preincubated for 30 min at 37 C in Krebs-Ringer bicarbonate buffer, pH 7.4, supplemented with 10 mmol/liter HEPES, 0.2% BSA, and 3.3 mmol/liter glucose as previously described (18). After preincubation (30 min), the buffer was changed to a medium of the same composition supplemented with the different test agents, and the islets were incubated for 60 min. All incubations were performed at 37 C in an incubation box (30 cycles/min). Each incubation vial was gassed with 95% O_2 and 5% CO_2 to obtain constant pH and oxygenation. Immediately after incubation, an aliquot of the medium was removed for assay of insulin (21). In the experiments with acarbose, islets were preincubated (with or without acarbose) for 60 min (to allow for acarbose uptake) at 1 mmol/liter glucose (9). After preincubation, the buffer was changed to a medium containing 1 or 16.7 mmol/liter glucose with or without acarbose, and the islets were incubated for 60 min unless otherwise stated (9).

Determination of lysosomal enzyme activities and neutral α-glucosidase in liver tissue

Liver specimens were homogenized in an acetate-EDTA buffer as described above to yield a 2% liver homogenate. Assays of acid phosphatase (pH 4.5), β-glucuronidase (pH 5.0), *N*-acetyl-β-D-glucosaminidase (pH 5.0), and acid (pH 4.0) and neutral (pH 6.5) α-glucosidase were performed with methylumbelliferyl-coupled substrates as previously described (3). The procedures for determination of acid glucan-1,4-α-glucosidase at pH 4.0 with glycogen as substrate and of cathepsin D at pH 4.0 with hemoglobin as substrate have been described previously (3). Protein was determined with the Lowry method (26).

Statistics

Levels of significance between sets of data were assessed using Student's *t* test for unpaired data or, where applicable, ANOVA followed by Tukey-Kramer's multiple comparisons test.

Results

In vivo effects of glucose on insulin secretion

GK rats were injected iv with glucose (11.1 mmol/kg), and the dynamics of the insulin and glucose responses were recorded and compared with the responses in appropriate groups of Wistar control rats. Figure 1 (*upper panel*) shows that glucose-induced insulin secretion was greatly impaired in the GK rats. In fact, the glucose injection did not stimulate insulin release; instead, it brought about a significant decrease in basal insulin levels at 15 min ($P < 0.001$). This strong impairment of insulin release in the GK rat was reflected in a marked deterioration of the glucose tolerance curve in these

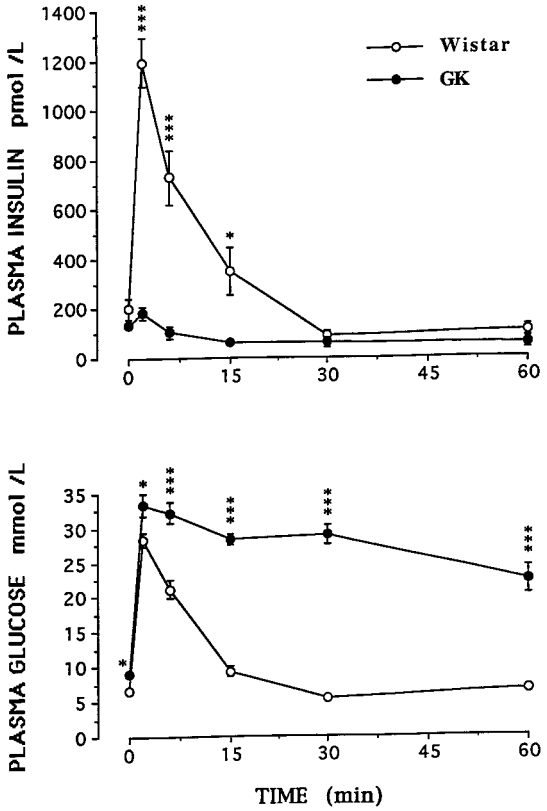

FIG. 1. Effect of an iv glucose load (11.1 mmol/kg) on the dynamics of insulin release and plasma glucose levels in Wistar control rats (Wistar) and NIDDM GK rats (GK). Values are the mean ± SEM for five rats in each group. Significant differences between Wistar controls and GK rats: *, $P < 0.05$; ***, $P < 0.001$.

rats (Fig. 1, *lower panel*). It should be noted that the basal plasma glucose levels (at time zero) were significantly increased in GK rats compared with those in Wistar control rats, whereas no difference was recorded with regard to the basal plasma insulin levels.

In vitro insulin secretion in response to glucose and forskolin

Figure 2 shows the effects of low (3.3 mmol/liter) and high (16.7 mmol/liter) glucose on insulin secretion from isolated islets of GK rats and Wistar control rats. It is seen that insulin secretion at low glucose (3.3 mmol/liter) was of similar magnitude in GK and Wistar islets. At high glucose, however, the Wistar control islets released almost 3 times more insulin than the GK islets during the 60-min incubation period. It is worth noting that in contrast to the absence of a significant insulin-releasing effect in the *in vivo* situation, the isolated GK islets responded to glucose stimulation *in vitro* by a slight, but significant, increase in insulin release (3.3 mmol/liter glucose, 0.30 ± 0.05 ng/islet·h *vs.* 16.7 mmol/liter glucose, 0.57 ± 0.08 ng/islet·h; $P < 0.05$). The addition of the adenylate cyclase activator forskolin to the incubation medium in the presence of 16.7 mmol/liter glucose dramatically increased insulin release from both Wistar and GK islets to the same level (Fig. 2).

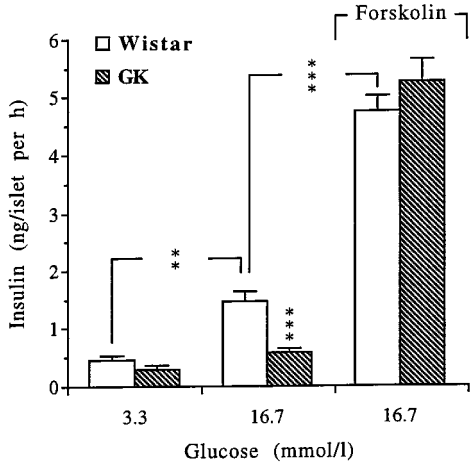

FIG. 2. Basal (glucose, 3.3 mmol/liter), glucose-stimulated (glucose, 16.7 mmol/liter), and forskolin (1 μmol/liter)- plus glucose (16.7 mmol/liter)-stimulated insulin release from islets isolated from Wistar control rats and GK rats. Significant differences between Wistar control islets and GK islets: ***, $P < 0.001$. Values are the mean ± SEM for 10–12 batches of islets in each group.

Lysosomal enzyme activities in isolated islets and liver tissue

Wistar controls and GK rats were analyzed for lysosomal enzyme activities in islet and liver tissue. Body weight, plasma concentrations of glucose and insulin, as well as islet insulin content of these rats are shown in Table 1. The GK rats displayed markedly increased plasma glucose concentrations, whereas the plasma insulin levels were similar in GK rats and Wistar controls. The islet insulin content was approximately 5.5 nmol/mg protein (60 ng insulin/islet) in both categories of rats. Figure 3 shows that compared with Wistar control rats, islets isolated from GK rats displayed decreased activities of the lysosomal enzymes acid phosphatase (−33%), *N*-acetyl-β-D-glucosaminidase (−25%), cathepsin D (−18%), and β-glucuronidase (−20%), whereas the activities of the two α-1,4-splitting glucoside hydrolases, acid glucan-1,4-α-glucosidase (+44%) and acid α-glucosidase (+37%), were increased. The activity of the neutral α-glucosidase (an enzyme attributed to the endoplasmic reticulum) was of the same magnitude in islets from GK and control rats.

Figure 4 shows the activities of the different lysosomal enzymes and the neutral α-glucosidase recorded in liver tissue from control and GK rats. The liver lysosomal enzyme activities displayed a pattern different from that of the islet enzymes. Thus, the activities of the α-1,4-splitting glucoside hydrolases were of similar magnitude in control and GK rats. The only significant difference with regard to lysosomal enzyme activities was recorded with β-glucuronidase, which was about 35% lower in the GK liver.

Phlorizin treatment

Groups of GK rats were treated with phlorizin or solvent (propylene glycol) for 9 days. A control group of Wistar rats treated with solvent was included. The mean body weights of the GK rats after the treatment period were 151 ± 8 g (phlorizin) and 142 ± 6 g (solvent). The Wistar rats (solvent) weighed 188 ± 7 g. The plasma glucose levels in the GK rats

TABLE 1. Metabolic status of young Wistar control and GK rats used in experiments in which islet and liver lysosomal enzyme activities were compared (Figs. 3 and 4)

	BW (g)	Plasma glucose (mmol/liter)	Plasma insulin (pmol/liter)	Islet insulin content (nmol/mg protein)
Wistar controls	223 ± 9.6	8.8 ± 0.7	199 ± 24	5.28 ± 0.60
GK rats	199 ± 5.8[a]	13.8 ± 0.9[b]	170 ± 32	5.52 ± 0.90

Values are the mean ± SEM for eight animals in each group.
[a] $P < 0.05$.
[b] $P < 0.001$.

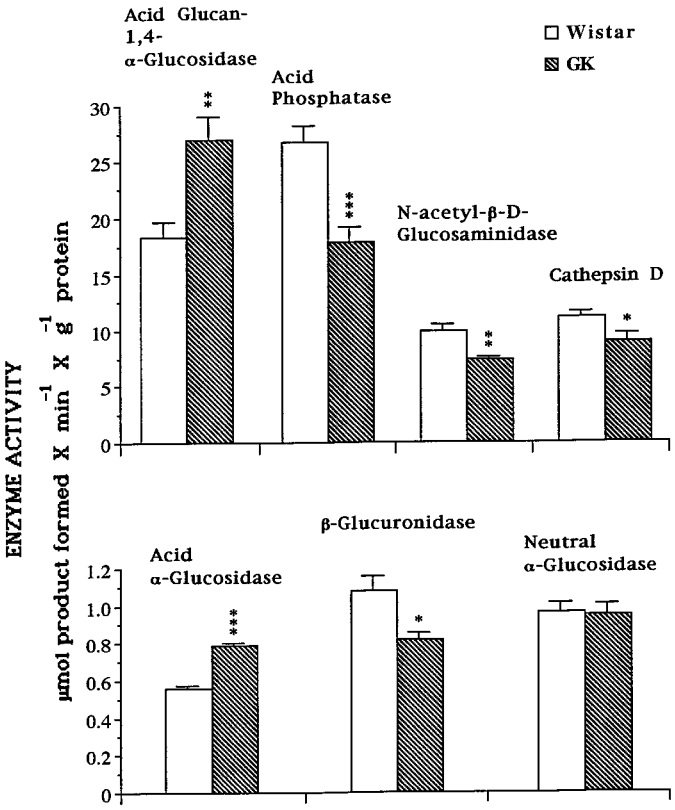

FIG. 3. Activities of different lysosomal enzymes and neutral α-glucosidase (endoplasmic reticulum) in isolated islets from Wistar control rats and GK rats, respectively. Enzyme activities are expressed as micromoles of glucose (acid glucan-1,4-α-glucosidase), tyrosine equivalents (cathepsin D), or 4-methylumbelliferone liberated per g protein/min. Values are the mean ± SEM for 200–300 islets isolated from each of 8 rats in the control and GK groups, respectively. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

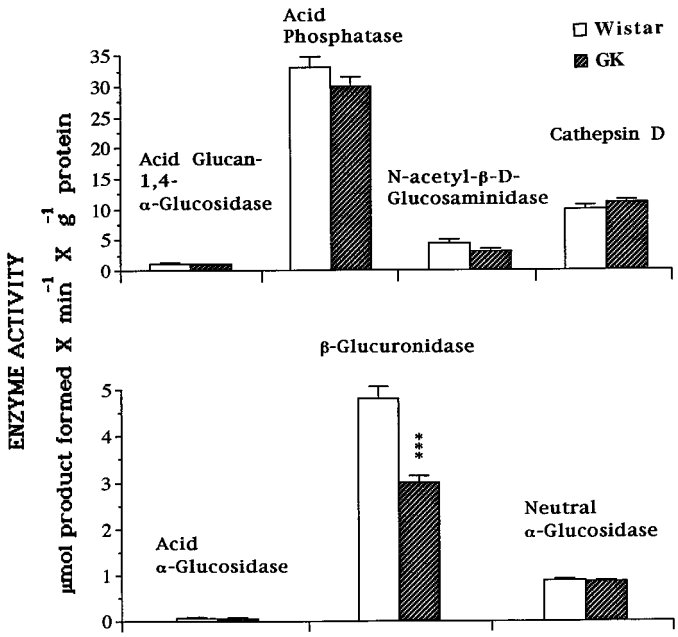

FIG. 4. Activities of different lysosomal enzymes and neutral α-glucosidase (endoplasmic reticulum) in liver tissue from Wistar control rats and GK rats, respectively. Enzyme activities are expressed as micromoles of glucose (acid glucan-1,4-α-glucosidase), tyrosine equivalents (cathepsin D), or 4-methylumbelliferone liberated per g protein/min. Values are the mean ± SEM for eight rats in each group. ***, $P < 0.001$.

at the end of the experiment were 8.8 ± 0.5 mmol/liter (phlorizin; n = 12) *vs.* 11.9 ± 1.0 mmol/liter (solvent; n = 9; $P < 0.01$). The plasma glucose levels in Wistar controls (solvent) were 7.6 ± 0.7 mmol/liter (n = 9). The corresponding plasma insulin levels were 145 ± 37 pmol/liter (GK, phlorizin) *vs.* 232 ± 33 pmol/liter (GK, solvent), and 228 ± 56 pmol/liter (Wistar, solvent). Islet lysosomal enzyme activities in the different groups of rats are illustrated in Fig. 5. Phlorizin treatment, in contrast to its normalizing effect on plasma glucose levels, did not normalize the different lysosomal enzyme activities in islet tissue of the GK rats. Figure 5 also shows that similar to the results obtained in the previous experiment (Fig. 3), the activities of acid glucan-1,4-α-glucosidase and α-glucosidase were markedly enhanced in GK rats compared with those in the Wistar controls, whereas the acid phosphatase and *N*-acetyl-β-D-glucosaminidase activities were again suppressed. The neutral α-glucosidase activity was of similar magnitude in all groups.

Experiments with the selective acid glucan-1,4-α-glucosidase inhibitor acarbose

To further test whether the GK islets might suffer from a general dysfunction of the islet lysosomal/vacuolar system, we performed a series of experiments with the pseudotetrasaccharide acarbose. Figure 6 shows the dose-response relationship for the effect of acarbose on lysosomal enzyme activities in islet homogenates as well as the effect on glucose-induced insulin release. Acid glucan-1,4-α-glucosidase and acid α-glucosidase activities were dose dependently inhibited by acarbose in islet homogenates from both Wistar and GK rats (Fig. 6, A and C), suggesting that the catalytic activities of the α-glucosidehydrolases in GK islet homogenates were unaffected. Other lysosomal enzyme activities in GK and Wistar islets were not influenced by acarbose, as ex-

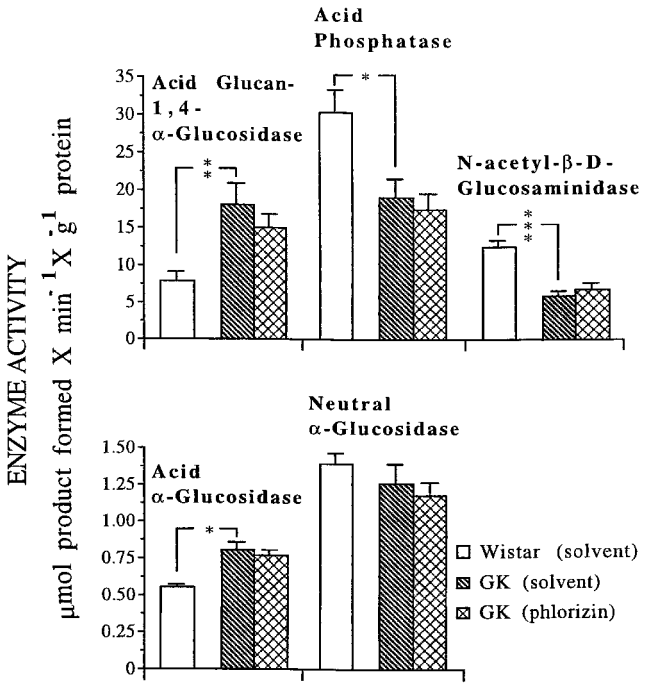

FIG. 5. Activities of different lysosomal enzymes and neutral α-glucosidase (endoplasmic reticulum) in isolated islets from Wistar control rats and GK rats, respectively. One group of GK rats (n = 12) was treated with phlorizin, and another (n = 9) was treated with solvent. The Wistar controls (n = 9) were treated with solvent. Enzyme activities are expressed as micromoles of glucose (acid glucan-1,4-α-glucosidase) or 4-methylumbelliferone liberated per g protein/min. Values are the mean ± SEM for 200–300 islets isolated from each rat. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

pected. Neutral α-glucosidase activity in islet homogenates was reduced at high concentrations of acarbose. Similar to the effects of acarbose on islet acid α-glucosidehydrolase activities, the glucose-stimulated insulin release from islets of Wistar controls was dose dependently suppressed by the pseudotetrasaccharide (Fig. 6B), whereas insulin release from GK islets was totally unaffected (Fig. 6D).

To directly asses the effect of acarbose on the lysosomal enzyme activities in intact islets and, thus, whether acarbose would have access to the islet lysosomal/vacuolar system, isolated islets (35 islets in 1.5 ml medium) from Wistar and GK rats were incubated at both low (1 mmol/liter) and high (16.7 mmol/liter) glucose in the presence of a high concentration of acarbose (10 mmol/liter). After incubation (120 min), aliquots of the incubation medium were removed for determination of insulin, and the islets were thoroughly washed and collected for assay of lysosomal enzyme activities (9). Figures 7 and 8 show that acarbose inhibited acid glucan-1,4-α-glucosidase activity at both low (modestly; Fig. 7A) and high (markedly; Fig. 8A) glucose in islets from Wistar control rats. Acid α-glucosidase activity was inhibited only at high glucose (Figs. 7C and 8C). In contrast, no effects of acarbose were observed on acid α-glucosidehydrolase activities in islets from GK rats (Fig. 7, A and C, and Fig. 8, A and C) suggesting that the lysosomal/vacuolar system itself, and not the acid α-glucosidehydrolase enzyme activities, was impaired. Other lysosomal enzyme activities or neutral α-glucosidase in Wistar or GK islets were not affected by acarbose (Fig. 7, D–F, and Fig. 8, D–F). Moreover, glucose-induced insulin release from GK islets was totally unaffected by this high concentration of acarbose, whereas glucose-induced insulin release from Wistar islets was severely impaired (Fig. 8B). Basal insulin release at low glucose was slightly inhibited by acarbose in Wistar islets, but was unaffected in GK islets (Fig. 7B). Finally, it should be noted that in accordance with the pattern of lysosomal enzyme activities in GK islets isolated directly *ex vivo* (Figs. 3 and 6), the present enzyme activities assayed in homogenates after incubation of intact islets *in vitro* also displayed increased acid α-glucosidehydrolase activities and decreased activities of other lysosomal enzymes at both low (Fig. 7) and high (Fig. 8) glucose compared with Wistar control islets.

Discussion

The present results show that young adult GK rats displayed significantly increased basal plasma glucose levels, whereas basal plasma insulin levels were not different from those in control rats. Increased plasma concentrations of both insulin and glucose, however, were seen in adult GK rats (18), suggesting that hyperglycemia precedes hyperinsulinemia. We show here that, similar to adult GK rats (18, 28), our young GK rats revealed a highly deteriorated glucose tolerance curve. This could be fully explained by the absence of an insulin secretory response to glucose at this early age. In fact, the iv glucose load in the GK rat brought about a significant decrease in insulin secretion. Such an impaired insulin response to glucose has previously been reported to occur in human NIDDM (29). This decreased insulin response to glucose in the GK rat is in agreement with previous observations in the *in vitro* perfused GK pancreas, where a tendency to an inhibitory response of insulin secretion was noted during perfusion with high glucose (16.7 mmol/liter) (18). In isolated GK islets the insulin response at 16.7 mmol/liter glucose was also markedly decreased, although somewhat less than those *in vivo* and in perfused pancreas. This is in accordance with previous studies in isolated islets from 2- to 3-month-old GK rats (18) and suggests that neural and/or circulating factors significantly contribute to the total absence of a glucose-induced insulin response *in vivo*.

In agreement with a previous study, forskolin greatly potentiated glucose-stimulated insulin release in normal Wistar islets and restored the glucose-induced insulin release in GK islets (30). Interestingly, forskolin was recently shown to overcome or compensate for the inhibitory action of emiglitate, a selective α-glucosidehydrolase inhibitor, on glucose-stimulated insulin secretion from isolated mouse islets (10). Hence, these data suggested that suppression of islet acid glucan-1,4-α-glucosidase activity and thus glucose-induced insulin release could be compensated for by a strong increase in the activity of the cAMP system. The present forskolin data also suggest that the exocytotic machinery *per se* in the GK islets is apparently unaffected.

The physiological significance of the lysosomal glycogenolytic hydrolases (acid α-glucosidehydrolases) in pancreatic β-cells is largely unknown. As mentioned above, previous studies (3–9, 12–14, 19) have disclosed an apparent relation between islet acid glucan-1,4-α-glucosidase activity and the

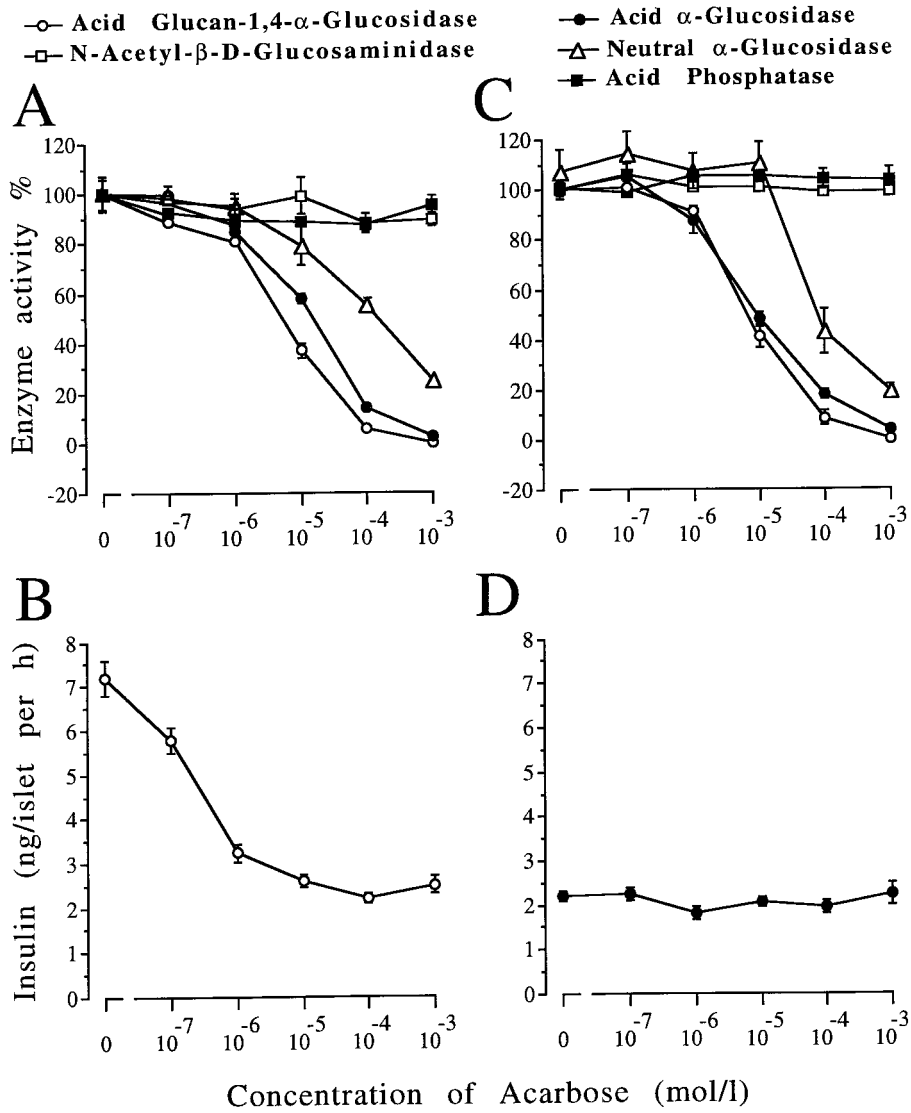

FIG. 6. Concentration-dependent effects of acarbose on enzyme activities in islet homogenates (A and C) as well as on glucose-stimulated (16.7 mmol/liter) insulin release from isolated islets (B and D) from Wistar (A and B) and GK (C and D) rats. The islet homogenates as well as the islets were preincubated with acarbose for 60 min before incubation started. Enzyme activities in homogenates are expressed as percentages of the control value. The mean ± SEM for 4 observations at each point are shown (A and C). Islets were isolated and pooled from at least 4 rats. Insulin release is expressed as nanograms of insulin secreted per islet/h. The mean ± SEM for 8–12 batches of islets pooled from at least 4 rats are shown (B and D).

insulin secretory response to glucose, but not to insulin-releasing agents, acting via direct stimulation of the adenylate cyclase and phospholipase C systems. In the present investigation we observed that the islets of the diabetic GK rat displayed significant abnormalities with regard to lysosomal enzyme activities both when assayed directly *ex vivo* and when analyzed after *in vitro* incubation of intact islets. Thus, the activities of acid glucan-1,4-α-glucosidase and acid α-glucosidase were markedly increased, whereas the activities of other lysosomal enzymes, such as acid phosphatase, *N*-acetyl-β-D-glucosaminidase, cathepsin D, and β-glucuronidase, were modestly decreased. It is possible that the intensity and duration of the hyperglycemia in our young GK rats could be of relevance for the elevation of the activities of the acid glucan-1,4-α-glucosidase and acid α-glucosidase, because hyperglycemia can be detected by the age of 1 week (31). As glucose utilization was shown to be 2- to 3-fold increased in GK rat islets (18, 32, 33), and exposure of isolated islets to high glucose *in vitro* augments the activity of acid lysosomal α-glucosidehydrolases (6, 7), it seems likely that the hyperglycemia itself and/or the increased rate of glycolysis are conceivable mechanisms contributing to the enhanced levels of the acid α-glucosidehydrolase activities in GK islets, although other mechanisms cannot be ruled out. However, not even an apparent normalization of the plasma glucose levels in the GK rat by phlorizin treatment for 9 days changed the elevated acid glucan-1,4-α-glucosidase activity. This is in accordance with our earlier observation that old, previously hyperglycemic, *ob/ob* mice that spontaneously returned close to normal plasma glucose levels still displayed markedly elevated levels of islet acid glucan-1,4-α-glucosidase activity (3, 13). However, in contrast to the GK rat, the *ob/ob* mouse did not display any abnormal function of the islet lysosomal system as such, as the elevated enzyme activity in this animal still correlated with the markedly increased plasma insulin levels (3, 13). Hence, although the islet acid glucan-1,4-α-glucosidase is significantly increased in the GK rat, it apparently is not given access to its substrate and consequently cannot exert its *in vivo* catalytic activity and mediate appropriate signaling in the glucose stimulus-secretion coupling. Therefore, it seems possible that it is not the enzyme itself but, rather, a malfunctioning lysosomal/vac-

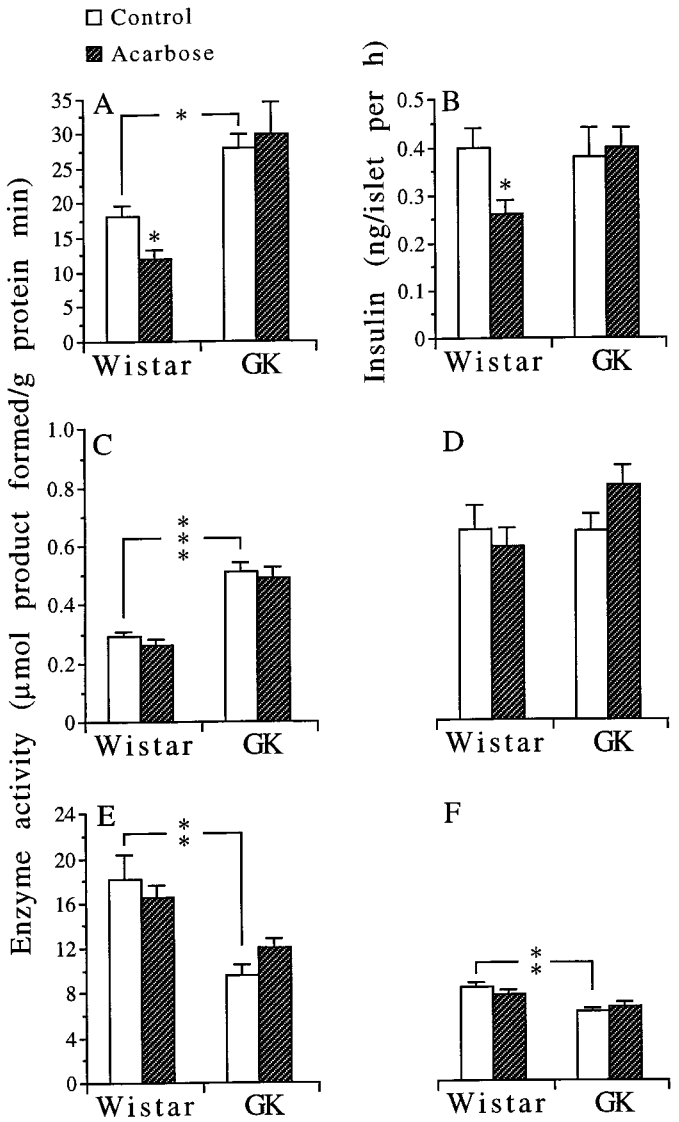

FIG. 7. Effect of 10 mmol/liter acarbose on activities of different lysosomal enzymes (A, acid glucan-1,4-α-glucosidase; C, acid α-glucosidase; E, acid phosphatase; F, *N*-acetyl-β-D-glucosaminidase) and on neutral α-glucosidase (D) and insulin release (B) in Wistar and GK islets incubated in 1 mmol/liter glucose for 120 min. Enzyme activities are expressed as micromoles of glucose (acid glucan-1,4-α-glucosidase) or 4-methylumbelliferone liberated per g protein/min. The mean ± SEM for six to eight batches of islets, each isolated from a single rat, are shown. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

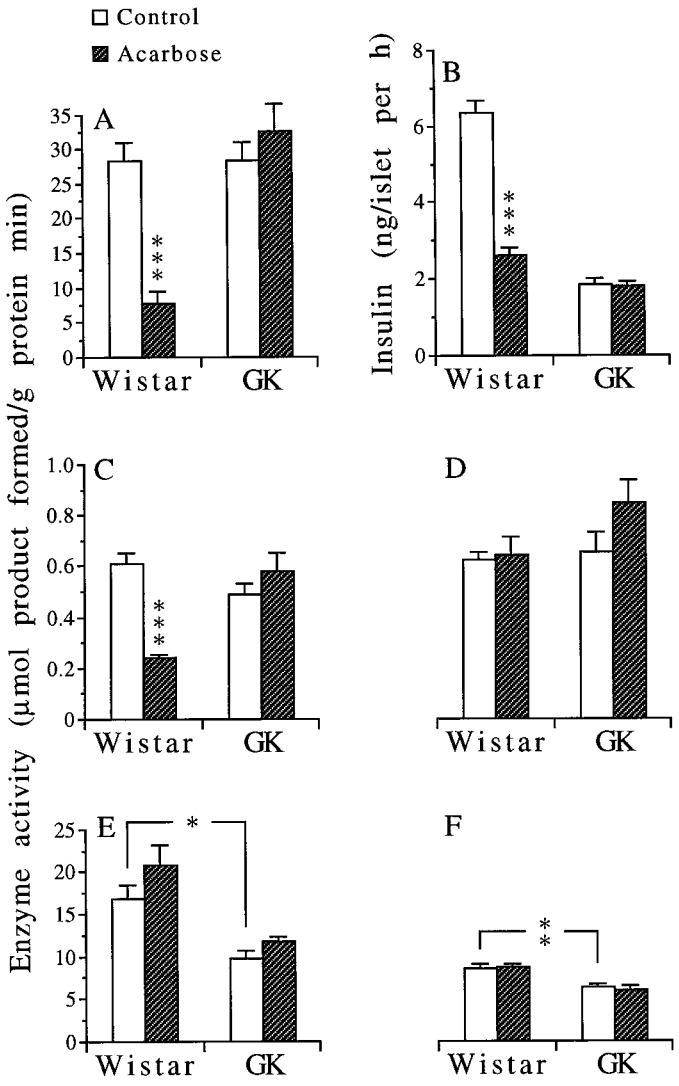

FIG. 8. Effect of 10 mmol/liter acarbose on activities of different lysosomal enzymes (A, acid glucan-1,4-α-glucosidase; C, acid α-glucosidase; E, acid phosphatase; F, *N*-acetyl-β-D-glucosaminidase) and on neutral α-glucosidase (D) as well as insulin release (B) in Wistar and GK islets incubated in 16.7 mmol/liter glucose for 120 min. Enzyme activities are expressed as micromoles of glucose (acid glucan-1,4-α-glucosidase) or 4-methylumbelliferone liberated per g protein/min. The mean ± SEM for six or seven batches of islets, each isolated from a single rat, are shown. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

uolar system as such that is an important and decisive feature of the impairment of glucose-induced insulin release in these rats. Further, the reduced activities of other lysosomal enzymes in the islets of the GK rat are also suggestive of an impaired function and activity of the whole lysosomal/vacuolar system. In contrast, the hepatic lysosomal enzyme activities, except β-glucuronidase, were not different from those in the control Wistar rats. The putative importance of the acid α-glucosidehydrolases in islet tissue is further underlined by the observation that in the control rats, the specific activities of islet acid glucan-1,4-α-glucosidase and acid α-glucosidase were approximately 15-fold higher than those in the liver, whereas all other enzyme activities were of generally the same magnitude in both islet and liver tissue. It should also be noted that the present finding of decreased activities of most lysosomal enzymes in the islets of our young GK rats is probably not a sign of catabolic and degenerative processes in these islets, as it is well known that degenerative changes in other tissues bring about increased levels of classical lysosomal enzyme activities, such as acid phosphatase, cathepsin D, and *N*-acetyl-β-D-glucosaminidase (34). Moreover, in our young GK rats the islet content of insulin is normal (*cf.* Table 1), and the morphological appearance of the islets is not different from that in normal control rats (35).

Finally, our data from the acarbose experiments provide further evidence to assume that in GK islets, it is not the

α-glucosidehydrolase activity *per se*, but the whole lysosomal/vacuolar system that is malfunctioning. We have previously shown (9) that the pseudotetrasaccharide acarbose, a potent inhibitor of islet acid α-glucosidehydrolase (9), when incubated together with mouse islets was taken up and exerted a strong inhibitory effect on both acid glucan-1,4-α-glucosidase activity and glucose-induced insulin release. In contrast, the tetrasaccharide maltotetraos, which is devoid of inhibitory properties and is a close analog of acarbose, did not affect either parameter (9). Additionally, preliminary *in vitro* experiments in our laboratory have shown that changes in glucose-stimulated insulin secretion and enzyme activities induced by α-glucosidehydrolase inhibition correlate temporally. The present data further revealed that with Wistar control islets the inhibition curve for glucose-stimulated insulin release in the presence of acarbose was dose dependent and similar in shape to the inhibition curve for acid glucan-1,4-α-glucosidase activity. However, it should be noted that the inhibition curve for insulin release was somewhat shifted to the left compared with the enzyme inhibition curve. These data speak in favor of a specific action of acarbose exerted on the acid glucan-1,4-α-glucosidase activity within the vacuolar system in the intact Wistar islets, as the concentration of an endocytosed compound such as acarbose, which is restrained within the vacuolar system, is always greater than that of a uniformly distributed compound (36). In contrast, no inhibitory effect by acarbose was recorded in intact, incubated islets from GK rats, showing that acarbose was not given access to its target, the acidic vacuolar compartment, in the β-cells. Moreover, our observation that the catalytic activities of acid glucan-1,4-α-glucosidase and acid α-glucosidase were suppressed to the same extent after direct addition of acarbose to islet homogenates of both GK and Wistar rats strongly speaks against a defect in acid glucan-1,4-α-glucosidase activity itself. An impaired function of the whole lysosomal/vacuolar system in intact GK islets is further underlined by the fact that no sign of an acarbose effect on α-glucosidehydrolase activities or insulin secretion was seen even if we used an extremely high concentration of acarbose (10 mM) in some experiments. Accordingly, as the catalytic activities of the α-glucosidehydrolases in GK islet homogenates indeed are not impaired, one would have expected, similar to the results of the Wistar control experiments, a clear and significant inhibitory action by acarbose on both of these enzyme activities as well as on glucose-induced insulin release in intact, incubated GK islets if the lysosomal/vacuolar system had been operating in a proper way. Hence, the intimate details of the pathophysiological mechanisms behind the dysfunction of the lysosomal/vacuolar system in the GK islets will await further investigations. Moreover, whether this dysfunction is somehow coupled to other previously reported defects in GK islet metabolism and ionic channel regulation (18, 30–33, 37–39) has yet to be elucidated.

In conclusion, we have shown that the secretory response to glucose was markedly impaired both *in vivo* and in isolated islets of the young GK rat. The defective response to glucose, however, could be compensated for in the presence of the adenylate cyclase activator forskolin. Further, in the GK rat the lysosomal enzyme activities in islet tissue (but not in liver tissue) showed an abnormal pattern, which could not be corrected by 9 days of phlorizin treatment and an apparent normalization of the plasma glucose levels. Moreover, the lysosomal/vacuolar system in the GK islets was not accessible to the pseudotetrasaccharide acarbose, suggesting a defective interaction between different organelle constituents within the vacuolar system. Whether this abnormality is a reflection of a general dysfunction of essential lysosomal membrane components and/or a deficiency of certain key factor(s) assisting lysosomal enzymes in their catalytic function remains to be elucidated. From the present and previous data, we therefore propose that the defective insulin response to glucose in the GK rat involves an important dysfunction of the islet lysosomal/vacuolar system, which prevents the acid glucan-1,4-α-glucosidase from exerting its action, and not an impairment of the acid glucan-1,4-α-glucosidase activity *per se*.

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