β-Cell Insensitivity to Glucose in the GK Rat, a Spontaneous Nonobese Model for Type II Diabetes

BERNARD PORTHA, PATRICIA SERRADAS, DANIELLE BAILBÉ, KEN-ICHI SUZUKI, YOSHIO GOTO, AND MARIE-HÉLÈNE GIROIX

In early 1988, a colony of GK rats was started in Paris with progenitors issued from F_{35} of the original colony reported by Goto and Kakisaki. When studied longitudinally up to 8 mo, GK rats showed as early as 1 mo (weaning) significantly higher basal plasma glucose (9 mM) and insulin levels (doubled), altered glucose tolerance (intravenous glucose), and a very poor insulin secretory response to glucose in vivo compared with Wistar controls. Males and females were similarly affected. Studies of in vitro pancreatic function were carried out with the isolated perfused pancreas preparation. Compared with nondiabetic Wistar rats, GK rats at 2 mo showed a significantly increased basal insulin release, no insulin response to 16 mM glucose, and hyperresponse to 19 mM arginine. Pancreatic insulin stores were only 50% of that in Wistar rats. Perfusion of GK pancreases for 50 or 90 min with buffer containing no glucose partially improved the insulin response to 16 mM glucose and markedly diminished the response to 19 mM arginine, whereas the responses by Wistar pancreases were unchanged. These findings are similar to those reported in rats with non-insulin-dependent diabetes induced by neonatal streptozocin administration and support the concept that chronic elevation in plasma glucose may be responsible, at least in part, for the β-cell desensitization to glucose in this model. The GK rat seems to be a valuable model for identifying the etiology of β-cell desensitization to glucose. *Diabetes* 40:486–91, 1991

The GK rat is a new spontaneous model of non-insulin-dependent (type II) diabetes without obesity that has been progressively characterized by Goto et al. (Tohoku Univ., Sendai, Japan; 1–3). This spontaneous diabetic state was produced by selective breeding repeated over numerous generations with glucose intolerance as a selection index and starting from a nondiabetic Wistar rat colony. Briefly, 18 rats were selected in 1973 from the local original Wistar stock (211 rats) with slight glucose intolerance, according to values of a standardized oral glucose test, and mated. This procedure was repeated over several generations. All offspring had a diabetic glucose tolerance test after F_{10} (2), and the diabetic state became stable after F_{30} (3). In 1988, we initiated a colony of GK rats in Paris, starting with progenitors issued from F_{35} of the Japanese colony.

This study was undertaken to obtain information concerning *1*) the longitudinal characteristics of the GK rat model when raised under our breeding conditions, *2*) the extent of the impairment of the secretory response of the β-cells in these rats, and *3*) the role of the chronically hyperglycemic environment in the GK rats on the functional impairment of their β-cells.

RESEARCH DESIGN AND METHODS

Five pairs of GK rats (F_{35} males and females) were obtained from Tohoku University School of Medicine in early 1988. Before mating was begun, all animals were mildly hyperglycemic when checked in the basal nonfasted state (10.9 ± 0.8 and 9.5 ± 0.9 mM, respectively, in males and females), and none were glycosuric. They were raised in parallel to nondiabetic Wistar rats obtained from a local supplier that were used as control rats (mean plasma glucose level in basal nonfasted state 6.7 ± 0.1 and 6.6 ± 0.1 mM, respectively, in males and females). All rats were fed ad libitum with a commercial pelleted chow (diet 113, Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France), and they were weaned 28 days after birth.

Evolution of body weight, basal plasma glucose and insulin levels, and pancreatic insulin content was followed in male rats until 32 wk of age. The male and female GK rats

From the Laboratory of Developmental Physiology, National Center for Scientific Research, University of Paris, Paris, France; and the Third Department of Internal Medicine, Tohuku University School of Medicine, Sendai, Japan.

Address correspondence and reprint requests to Dr. Bernard Portha, Laboratoire de Physiologie du Développement, CNRS URA 307, Université Paris 7, Tour 33, 2 Place Jussieu, 75251 Paris, France.

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used in this study were issued from F_2 to F_4 of our GK colony. Their characteristics were stable throughout the generations.

Glucose tolerance tests (0.5 g/kg i.v.) were performed in GK and control male and female rats at 8, 20, or 32 wk of age. The tests were performed at 1400 in rats fasted from 0900 under pentobarbital sodium anesthesia (4 mg/100 g body wt i.p.). Blood was withdrawn from the tail vein. Blood samples (300 μl) were immediately centrifuged at 4°C, and plasma was stored at −20°C until assayed.

For the isolated pancreas-perfusion technique, 2- to 3-mo-old male GK or Wistar rats were anesthetized with pentobarbital sodium (4 mg/100 g body wt i.p.). Isolation and perfusion of the pancreas were performed as previously described (4). The perfusate was a Krebs-Ringer bicarbonate buffer with the following components: 118 mM NaCl, 4 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 1.25 g/L fatty acid–free bovine serum albumin (Sigma, St. Louis, MO), and 40 g/L dextran 70 (Pharmacia, Uppsala, Sweden). D-Glucose (Merck, Darmstadt, Germany) or L-arginine (Sigma) were administered through a sidearm syringe as required. The complete effluent (3 ml/min) was collected from the cannula in the portal vein at 1-min intervals in chilled tubes and stored at −20°C until assay. After perfusion, the pancreas was weighed, homogenized, and centrifuged at 4°C in an acid-alcohol solution (75% ethanol, 1.5% HCl 12 mM, and 23.5% distilled H_2O). The supernatant was stored at −20°C until assayed.

Analysis. Plasma glucose was determined with a glucose analyzer (Beckman, Fullerton, CA). Immunoreactive insulin in the perfusate and pancreases was estimated with purified rat insulin as standard (Novo, Copenhagen) and antibodies to mixed (pork and beef) insulin and pork [^{125}I]monoiodinated insulin (5). Charcoal was used to separate free from bound hormone. The method allows the determination of 0.12 ng/ml, with a coefficient of variation within and between assays of 10%.

Insulin and glucose responses during the glucose tolerance tests were calculated as the values of incremental plasma insulin areas integrated over 30 min after the injection of glucose (ΔI) and the corresponding incremental integrated plasma glucose areas (ΔG). The rate of glucose disappearance (K_d) was calculated from the slope of the regression line obtained with the log-transformed plasma glucose values between 5 and 30 min after glucose administration.

Insulin secretion rate per total pancreas was calculated by multiplying the insulin concentration in the samples by the flow rate. Total insulin response to glucose or arginine was obtained by planimetry of the individual perfusion profiles and expressed as the difference in hormonal secretion rate relative to the mean hormonal output recorded at the end of the prestimulation period. Results are given as means ± SE. Statistical analyses were performed with Student's *t* test for unpaired data.

RESULTS

Development of diabetic state in GK rats. Growth curve of the GK rats was close to that of the nondiabetic Wistar rats (Fig. 1). However, body weights were significantly decreased ($P < 0.01$) from 8 to 32 wk of age in the male GK rats.

As early as 4 wk after birth (i.e., just before weaning), male GK rats exhibited significant hyperglycemia (Fig. 1). When rats were adults and up to 32 wk of age, the basal plasma glucose levels measured in the postabsorptive state (6 h of fasting) remained moderately elevated (9.5 ± 0.3 mM at 32 wk) and significantly higher ($P < 0.001$) in the GK rats compared with controls (Fig. 1). Over this period of observation, there was no indication of deterioration of glycemic status in the GK rats. Such a moderate hyperglycemia was detectable in all F_1 and F_2 animals of both sexes obtained from the starting pairs of progenitors.

The pancreatic insulin contents were sharply decreased at 10 wk in the male GK rats, representing only 50% of the related control stores (Fig. 1). At 8 wk of age, basal plasma insulin values in the GK males and females were significantly higher (162 and 159% of control values, respectively, $P < 0.001$; Table 1) and remained significantly higher at 20 and 32 wk (274 and 172% in males and 169 and 152% in females, respectively).

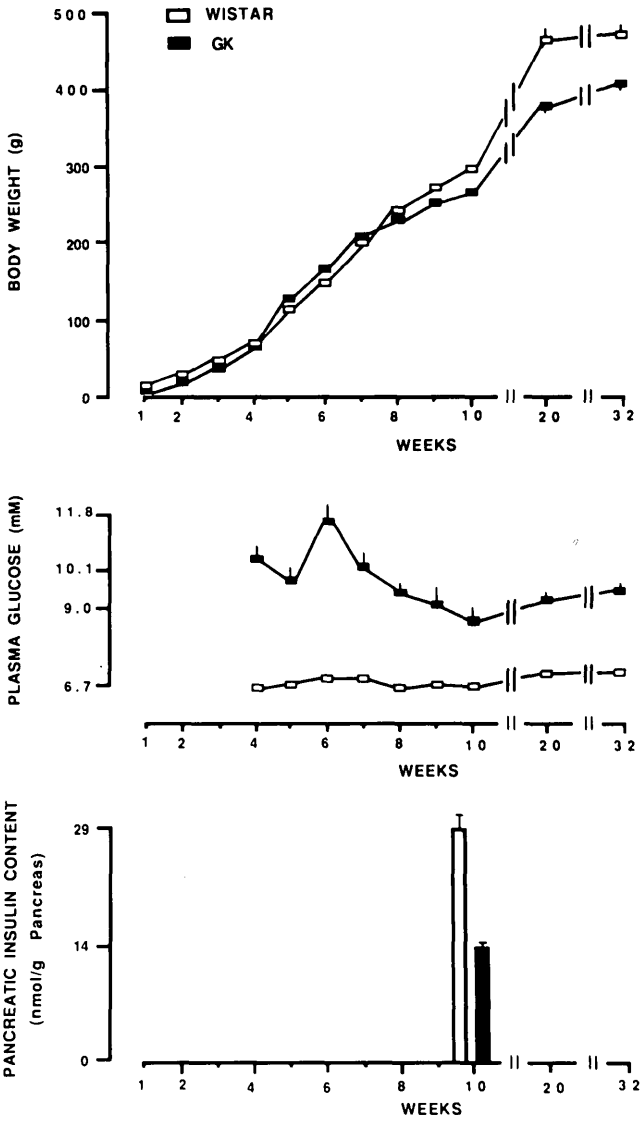

FIG. 1. Evolution of body weight, basal plasma glucose level, and pancreatic insulin stores in male nondiabetic Wistar and diabetic GK rats from 1 to 32 wk of age. *Points*, means ± SE of 10–15 observations/group.

TABLE 1
Body weight, basal plasma glucose and insulin levels, and ΔG, K_d, and ΔI in 8-wk-old GK and Wistar (control) rats

	n	Weight (g)	Plasma glucose (mM)	Plasma insulin (pM)	ΔG (mM · min)	K_d (%)	ΔI (nM · min)
Males							
Wistar	10	244 ± 4	6.7 ± 0.1	675 ± 43	11.2 ± 0.5	1.96 ± 3.5	5.8 ± 0.3
GK	15	231 ± 2*	8.5 ± 0.2†	1091 ± 65†	14.5 ± 1.5	0.54 ± 0.08†	1.0 ± 0.3†‡
Females							
Wistar	18	171 ± 4	6.6 ± 0.1	740 ± 72§	14.6 ± 0.7	2.71 ± 0.23	6.8 ± 0.6
GK	16	154 ± 2†	8.5 ± 0.3†	1178 ± 43†	13.0 ± 1.3	0.78 ± 0.11†	2.9 ± 0.6†

Values are means ± SE. Mean incremental plasma glucose areas (ΔG), mean incremental insulin areas (ΔI), and the rate of glucose disappearance (K_d) were calculated from values obtained during 30-min glucose tolerance tests.
*$P < 0.01$, †$P < 0.001$, vs. same-sex Wistar.
‡$P < 0.02$ vs. female GK.
§$n = 16$.

After intravenous glucose, the values of ΔG were significantly increased ($P < 0.001$) in the 20- and 32-wk-old male GK rats compared with the control males (Fig. 2). The K_d and ΔI values were significantly decreased ($P < 0.001$) in the male GK rats at 8, 20, and 32 wk of age (Fig. 2).

Comparison between male and female GK rats was made at 8 wk of age (Table 1). No significant difference linked to sex could be detected concerning basal plasma glucose and insulin levels, K_d values, and ΔG values obtained during a standardized glucose tolerance test. However, ΔI values during the test were significantly lower ($P < 0.02$) in male than in female GK rats.

In vitro insulin secretion in GK rats. In vitro insulin release in response to glucose and arginine was studied with the isolated perfused pancreas preparation. Basal insulin secretion under conditions of no glucose in the basal perfusion medium was significantly increased in GK rats: secretion rate was 4.8 ± 1.4 vs. 0.8 ± 0.1 ng/min in nondiabetic Wistar rats ($n = 6$/group, $P < 0.02$). Exposure to 16 mM glucose after a 20-min basal period (our current procedure in pancreas-perfusion experiments), a concentration that induced a typical biphasic pattern of insulin release in control pancreases, did not elicit a clear-cut increase of insulin output in the GK pancreases (Fig. 3, *top*). The incremental insulin response to glucose was very low compared with control values (0.3 ± 0.2 vs. 11.48 ± 1.6 ng/min, $P < 0.001$, $n = 6$/group). More careful examination of the release pattern indicated a tendency toward a paradoxical decline of insulin secretion as a function of duration of exposure to 16 mM glucose. At cessation of glucose stimulation, a paradoxical rise in insulin release occurred.

We then evaluated the insulin response to glucose in GK rats when the pancreas was perfused for an extended period with a buffer containing no glucose. In control rats, during perfusion with a medium containing no glucose, the basal rate of insulin secretion was significantly decreased as a function of time (0.5 ± 0.1 ng/min at 20 min vs. 0.2 ± 0.1 ng/min at 50 min, $P < 0.05$, $n = 10$/group). After the 50-min glucose-omission period, integrated insulin response to subsequent stimulation with 16 mM glucose was not significantly different from that obtained after the 20-min glucose-omission period (Fig. 3). In GK rats, the basal rate of insulin release was significantly decreased during the 90 min of glucopenia (2.8 ± 0.4 ng/min at 20 min vs. 1.2 ± 0.1 ng/min at 50 min and 0.8 ± 0.2 ng/min at 90 min, $P < 0.01$, $n = 6$/group). After the 50-min glucose-omission period, GK rats exhibited a partial reversibility of the lesion, as evidenced by a consistent first phase of insulin release in response to glucose. This was confirmed by the experiment with the 90-min washout period (Fig. 3). The incremental insulin release in response to 16 mM glucose after the 50-min glucose-omission period in GK rats was increased 12-fold ($P < 0.001$) compared with that after the 20-min glucose-omission period. It was not further enhanced by the 90-min washout period. However, β-cells in GK rats under these conditions still lacked second-phase insulin release, and a paradoxical insulin response to the switch from high to low glucose persisted (Fig. 3). The insulin response to 19 mM arginine tested after the 20-min glucose-omission period (our current procedure) was increased 18-fold ($P < 0.001$) in GK rats compared with nondiabetic rats (Fig. 4, *top*). This hyperresponse to arginine in GK rats was partially corrected after the 50- and 90-min washout periods. After the 50- and 90-min glucose-omission periods, the insulin secretory response to 19 mM arginine was decreased by 38 and 76%, respectively, but the response measured after the 90-min washout period remained significantly higher (3-fold, $P < 0.02$) than the normal arginine response in Wistar rats.

DISCUSSION

GK rats provide a new genetic model of type II diabetes without obesity (1–3). The characteristics of animals bred in our colony are close to those of the animals in the original colony (6): all F_1–F_4 rats obtained in our department had a basal mild hyperglycemia and an impaired intravenous glucose tolerance test. Males and females were similarly affected, and their diabetic state was stable over 32 wk of observation. In adult GK rats, pancreatic insulin stores were decreased by 50%, which is consistent with the decreased percentage of β-cells in the islets previously reported (3). Plasma insulin release in vivo in response to intravenous glucose was lacking, an observation consistent with the sluggish and delayed insulin response to oral glucose tolerance tests observed by Goto et al. (6). Because β-cell number and insulin stores in the pancreas of GK rats were low, the defective insulin response observed in vivo could be attributed to these quantitative abnormalities of the islets. In fact, additional alterations in β-cell responsiveness to some stim-

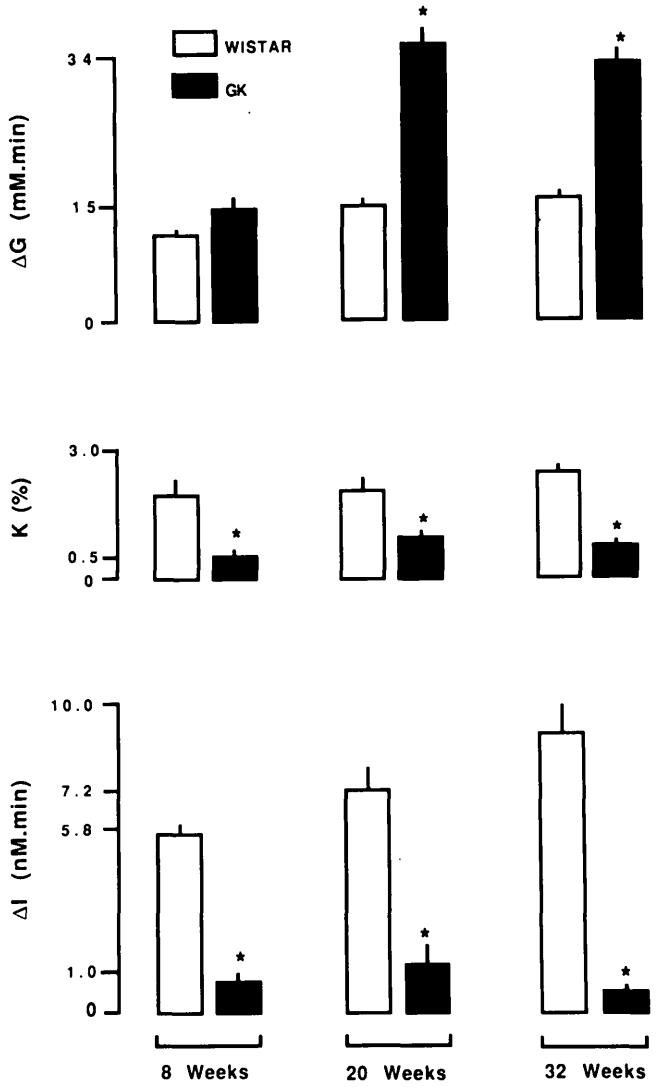

FIG. 2. Glucose tolerance and plasma insulin response to glucose (0.5 g/kg i.v.) in male nondiabetic Wistar and diabetic GK rats measured at 8, 20, and 32 wk of age. Mean incremental plasma glucose areas (ΔG), mean incremental insulin areas (ΔI), and glucose disappearance rates (*K*) were calculated from data obtained during 30-min glucose tolerance tests. *Bars*, means ± SE of 10–15 observations/group. *$P < 0.001$ vs. age-matched Wistar group.

uli were present. In vitro studies of insulin release with the isolated perfused-pancreas technique indicated a lack of response to glucose and a dramatic hyperresponse to arginine when this stimulus was given in the absence of glucose. Note that an impaired insulin-response pattern was previously reported in F_9 GK rats in the Japanese colony (7).

The pattern of insulin release found herein is qualitatively similar to that previously described in adult rats receiving streptozocin at birth (n0-STZ model; 4) or on day 2 after birth (n2-STZ model; 8,9). It is also close to that reported in diabetic rats with partial pancreatectomy (10,11); a genetic model of type II diabetes, the SHR/N-*cp* rat (12); and BB rats at the onset of autoimmune insulin-dependent diabetes (13,14), and it is identical to that in which nondiabetic rats received glucose infusions for 48–96 h (15,16).

To examine the role of hyperglycemia per se on the two major defects in insulin secretion, perfusion of GK pancreases were conducted with glucose-free medium for 50 or 90 min. Under this protocol, insulin response to high glucose was partially improved. The last finding agrees with that obtained in both n0-STZ (P.S., B.P., unpublished observations) and n2-STZ (17) Wistar rats and SHR/N-*cp* rats (12) with similar in vitro glucopenia protocols. The mechanism behind this effect remains unknown. The effect of glucose omission seemed specific for hyperglycemic rats because glucose omission failed to exert a significant effect on the subsequent response to glucose in pancreases from control rats. One possible explanation for the improvement of glucose-induced insulin secretion by glucose-free buffer is that an

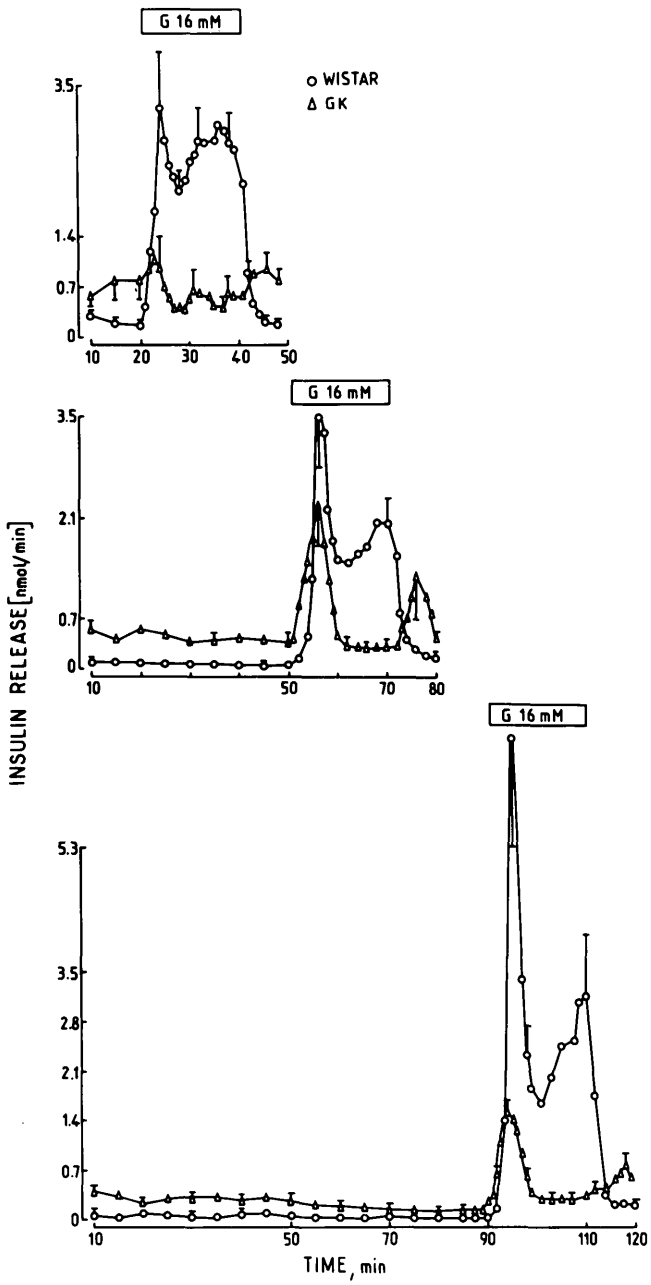

FIG. 3. Effect of 16 mM glucose on insulin release from perfused pancreas of male nondiabetic Wistar and diabetic GK rats. Glucose was omitted from basal perfusion medium between min 0 and 20 (*top*), 0 and 50 (*middle*), or 0 and 90 (*bottom*) and after stimulatory period. *Points*, means ± SE of 3–10 observations/group.

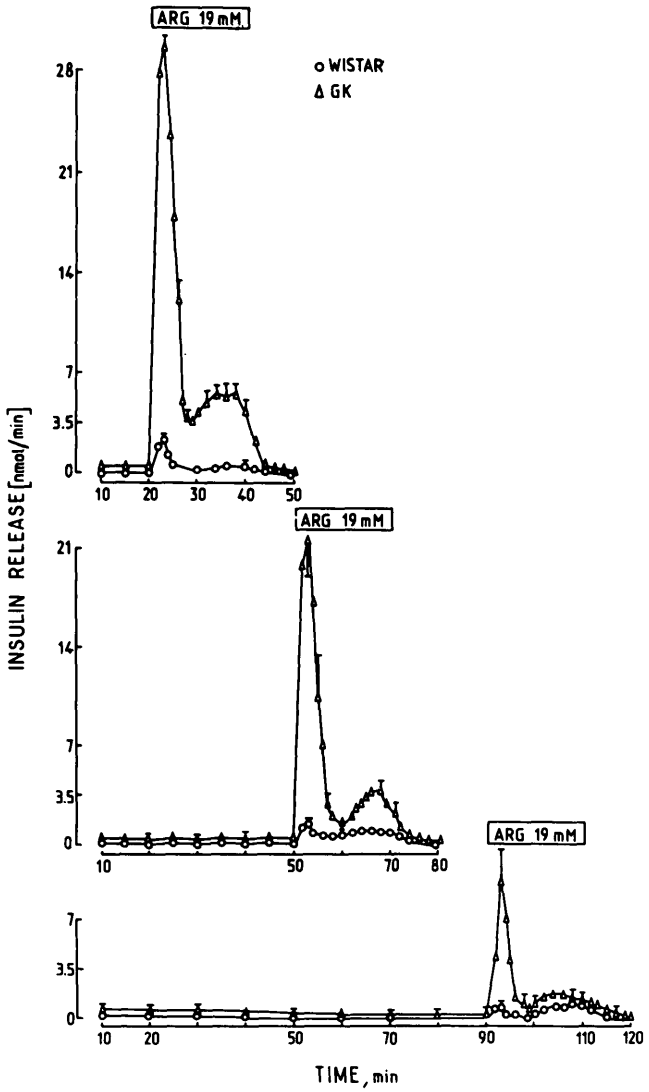

FIG. 4. Effect of 19 mM arginine on insulin release from perfused pancreas of male nondiabetic Wistar and diabetic GK rats. Glucose was omitted throughout perfusion (including period of arginine stimulation) between min 0 and 20 (*top*), 0 and 50 (*middle*), or 0 and 90 (*bottom*) and after stimulatory period. *Points*, means ± SE of 3–8 observations/group.

inhibitory component released in high glucose is washed out from the diabetic pancreas. However, this hypothesis is difficult to reconcile with the observation that the hyperresponse to arginine is indeed decreased under the same glucopenic conditions.

The second aspect of our study was related to the etiology of the hyperresponsiveness of β-cells to arginine in the GK rat model. Because the effect of arginine was approaching maximum at low glucose concentrations and the β-cells were acting as if they were already potentiated, this abnormality can be considered the failure of arginine-stimulated insulin secretion to be turned off appropriately by low glucose (11). Similar conclusions have also been reported in n0-STZ Wistar rats (18), n2-STZ Sprague-Dawley rats (11), and SHR/N-*cp* rats (12), and it has been suggested that the chronic hyperglycemia was responsible for this effect (8,9,18). This conclusion can now be extended to GK rats, because omission of glucose in the perfusion buffer was associated with markedly diminished response to arginine by GK rat pancreases, whereas the response to arginine in control rats was unchanged. The mechanisms behind an enhanced response to nonnutrient secretagogue in GK rats can only be speculated on in the absence of any data related to GK rat isolated islets. Availability of endogenous substrates could positively regulate the insulin secretory response to nonnutrient stimuli when tested in the absence of exogenous fuels. Also, diabetes (19) or short-term hyperglycemia (20,21) leads to increased amounts of glycogen in islets, glycogen stores are rapidly depleted by fuel deprivation (20), and high glycogen stores in the islets are associated with increased responsiveness to nonnutrient stimuli (22). Hence, increased levels of endogenous substrates, in the form of glycogen or not, probably underlie the enhanced response to arginine in GK rats, and glucose omission could decrease a hyperresponse to the amino acid by decreasing the levels of endogenous substrates.

In conclusion, the GK rat is a genetically diabetic nonobese model of human diabetes that shows alterations in pancreatic function that closely resemble those induced by nO-STZ in rats (8,23). One potential weakness of the nO-STZ compared with the GK rat model is that it is difficult to definitively exclude the possibility that the pathogenesis of β-cell desensitization to glucose is related, at least in part, to a permanent toxic action of STZ. Indeed, after an STZ-induced injury to adult islets in vitro, the surviving β-cells are able to maintain most of their metabolic functions but fail to maintain adequate insulin production (24,25). Thus, GK rats seem more appropriate for identifying the etiology of β-cell desensitization to glucose. According to our data, chronic elevation in plasma glucose may be responsible for the glucose desensitization of β-cells of GK rats. However, this important assumption needs to be confirmed by testing diverse in vivo maneuvers aimed to chronically lower the plasma glucose levels.

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