The GK rat model of type 2 diabetes is especially convenient to dissect the pathogenic mechanism necessary for the emergence of overt diabetes because all adult rats obtained in our department (GK/Par colony) to date have stable basal mild hyperglycemia and because overt diabetes is preceded by a period of normoglycemia, ranging from birth to weaning. The purpose of this article is to sum up the information so far available related to the biology of the β-cell in the GK/Par rat. In terms of β-cell function, there is no major intrinsic secretory defect in the prediabetic GK/Par β-cell, and the lack of β-cell reactivity to glucose (which reflects multiple intracellular abnormalities), as seen during the adult period when the GK/Par rats are overtly diabetic, represents an acquired defect (perhaps glucotoxicity). In terms of β-cell population, the earliest alteration so far detected in the GK/Par rat targets the size of the β-cell population. Several convergent data suggest that the permanently reduced β-cell mass in the GK/Par rat reflects a limitation of β-cell neogenesis during early fetal life, and it is conceivable that some genes among the set involved in GK diabetes belong to the subset of genes controlling early β-cell development. Diabetes 50 (Suppl. 1):S89–S93, 2001

Type 2 diabetes develops as a consequence of interplay among β-cell dysfunction, peripheral insulin resistance, and elevated hepatic glucose production. However, it is not known which is the primary abnormality and which are abnormalities secondary to elevated plasma glucose, so-called glucose toxicity. To delineate the primary abnormalities, it is desirable to analyze individuals destined to become diabetic before the development of the disease. The advantage of using an animal model is that the development of diabetes can be predicted and thus it is possible to dissect the pathogenic mechanism necessary for the emergence of overt diabetes. The Goto-Kakizaki Wistar rat (GK rat) is especially useful because all adult animals of both sexes exhibit type 2 diabetes. This spontaneous diabetes model was produced by selective breeding (with glucose intolerance as a selection index) repeated over many generations, starting from a non-diabetic Wistar rat colony. The characteristics of GK animals bred in our colony in Paris (GK/Par) for more than 10 years (1) are very stable and remain close to those of the animals in the original Japanese colony (2): all of the rats have a basal mild hyperglycemia and impaired glucose tolerance. Males and females are similarly affected, and their diabetic state is stable over 72 weeks of follow-up (3). In adult GK rats, plasma insulin release in vivo in response to intravenous glucose is abolished (1,3). In vitro studies of insulin release with the isolated perfused pancreas (1) or with perfused islets (4) indicate that both early and late phases of glucose-induced insulin release are markedly affected in the adult GK rat. Concerning insulin action in adult GK rats, we have reported decreased insulin sensitivity in the liver, in parallel with moderate insulin resistance in extrahepatic tissues, i.e., muscle and adipose tissue (5,6). In our colony (GK/Par), hyperglycemia is preceded by a period of normoglycemia, ranging from birth to weaning (7). Therefore, during this period, young GK rats can be considered to be prediabetic.

DECREASED β-CELL NUMBER AND MULTIPLE β-CELL FUNCTIONAL DEFECTS IN THE ADULT GK/Par RAT WITH OVERT DIABETES

In the adult GK rat, total pancreatic β-cell mass is decreased (by 60%) in the range of the decrease in pancreatic insulin stores (1,7,8) (Fig. 1). This alteration of the β-cell population cannot be ascribed to increased β-cell apoptosis but is related, at least partly, to significantly decreased β-cell replication (Fig. 1). Moreover, the adult GK pancreas exhibits two different populations of islets: large islets, which are disrupted by connective tissue (7) and display heterogeneity in the staining of the β-cells, and small islets, with heavily stained β-cells and normal architecture (Fig. 1).

The islets of adult GK/Par rats, at least after collagenase digestion, show decreased β-cells and low insulin content compared with control islets. The islet DNA content was decreased to a similar extent; this is consistent with our morphometric data (Fig. 2), which indicate that there is no major change in the relative contribution of β-cells to total endocrine cells in the GK islets. In addition, in GK islets,
insulin content, when expressed relative to DNA, remains lower than in control islets, which supports degranulation in the β-cells of diabetic animals.

The notion that in the GK β-cell the lesion responsible for loss of glucose-induced insulin secretion is mostly upstream of the effector system is supported by data indicating that GK islets are only responsive to nonnutrient secretagogues, such as sulfonylureas or a combination of barium and theophylline (9). We have reported that glucose transport and glucose phosphorylating activity are not modified in the GK β-cells (4,10). Consistent with these conclusions is the present observation that the expression of glucose transporter GLUT2 (by reverse transcriptase–polymerase chain reaction) is normal in GK islets (Fig. 3). It is also noteworthy that in GK (as well as Wistar) rat islets, we were unable to detect expression of glucose-6-phosphatase (whereas in GK rat liver, glucose-6-phosphatase was easily detected) (Fig. 3). This is an interesting point because in another GK colony (11), it has been reported that islet glucose-6-phosphatase activity was increased, as was cycling between glucose and glucose-6-phosphate (11).

We have shown that impaired glucose-induced insulin release in GK islets is associated with perturbation of multiple mitochondrial functions. More specifically, we reported that aerobic, but not anaerobic, glycolysis is impaired in GK islets (4,9,10), and we showed that mitochondria of GK islets exhibit a specific decrease in the activities of flavin adenine dinucleotide–dependent glycerolphosphate dehydrogenase (4,10) and branched-chain ketoacid dehydrogenase (12). Although this certainly may contribute to lower oxidation rates, it does not exclude other mechanisms. Indeed, we found that the β-cells of adult GK rats had a significantly smaller mitochondrial volume than control β-cells (13). No major deletion or restriction fragment polymorphism could be detected in mtDNA from adult GK islets (13); however, they contained markedly less mtDNA than did control islets. The lower islet mtDNA was paralleled by decreased content of some islet mt mRNAs, such as cytochrome b (13). In accordance with this, insufficient increase of ATP generation in response to high glucose was shown by our group (4) (Fig. 4). Finally, the impaired insulin response to glucose may be attributed to impaired elevation of intracellular Ca2+ concentration, which seems to be a consequence of the failure by glucose to augment L-type Ca2+ channel activity because of insufficient plasma membrane depolarization, reflecting impaired closure of ATP-sensitive K+ channels (Fig. 4). We have recently obtained data supporting the view that abnormal Ca2+ handling by the endoplasmic reticulum may also participate in defective Ca2+ signaling; we investigated the cytosolic calcium response to high glucose in single perfused GK islets, as measured by dual-wave spec-
trophotometry using fura-2 (14). The most prominent difference is detected in the first 5 min following high glucose, because the GK islet lacks the initial reduction of cytosolic calcium. This initial reduction is thapsigargin-sensitive in the normal islet, suggesting that the sequestration of calcium by endoplasmic reticulum, attributed to activation of calcium-ATPases (SERCAs), is impaired in the GK β-cell (Fig. 4). Such a conclusion is consistent with the report that SERCA3 gene expression is downregulated in GK islets (15). Alternatively, impaired calcium sequestration in the GK β-cell can also be accounted for by insufficient cytosolic ATP generation in response to high glucose (Fig. 4).

FIG. 2. Characteristics of collagenase-isolated islets from 4-month-old male GK/Par rats and control Wistar rats. Data are means ± SE. The number of islet preparations is between 3 (morphometric studies) and 18 (biochemical determinations). In each experiment, DNA and insulin content values were obtained from two to five groups of 20 islets each, and the percentages of β-cells and non-β endocrine cells (α, δ, and PP) were estimated in four to five islets. **P < 0.05, ***P < 0.001 compared with related value in control Wistar group.

FIG. 3. Detection of glucose-6-phosphatase (Glc6Pase) and GLUT2 mRNAs in islet and liver from 4-month-old male GK/Par rats and control Wistar (W) rats. Total RNAs were purified from freshly isolated islets and a frozen liver sample from GK and Wistar rats, using the Qiagen purification procedure. Glucose-6-phosphatase cDNA (1,095 bp, exons 1–5) was amplified from 1 µg total RNA in 50 µl amplification medium as previously described (25), and 20 µl was analyzed on 1.5% agarose gel. GLUT2 cDNA (808 bp, exons 6–11) was amplified from the same RNA samples and analyzed accordingly. Digestion products of phage-λ by HindIII/EcoRI were analyzed as size markers (DNA fragments appearing were 2,027, 1,904, 1,584, 1,330, 983, 832, and 564 bp long, as shown on the left of the figure from top to bottom).

FIG. 4. Model for defective glucose-induced insulin release in the GK/Par β-cell. Impaired insulin response to glucose may be attributed to impaired elevation of intracellular Ca2+ concentration, which seems to be a consequence of the failure by glucose to augment L-type Ca2+ channel activity, in its turn due to insufficient plasma membrane depolarization, reflecting impaired closure of the ATP-sensitive K+ channels; this is the result of insufficient cytosolic ATP generation by glucose. Abnormal Ca2+ handling by the endoplasmic reticulum (ER) in response to high glucose may also participate in the defective Ca2+ signaling: the sequestration of calcium by ER during high-glucose exposure (attributed to activation of the SERCAs) is impaired in the GK rat β-cell. Impaired calcium sequestration can also be accounted for by insufficient cytosolic ATP generation in response to high glucose: in GK islets, glucose fails to increase inositol triphosphate (IP3) accumulation. This is linked to an anomaly in targeting the phosphorylation of phosphoinositides: the activity of phosphatidylinositol kinase, the first of the two phosphorylating activities responsible for generating phosphatidylinositol biphosphate, is reduced. Moreover, deficient calcium handling and ATP supply in response to glucose probably also contribute to abnormal activation of phosphatidylinositol kinases and phospholipase C.
Finally, we investigated phosphoinositides (16) and cAMP metabolism (17) in GK islets. Whereas carbachol was able to promote normal inositol generation in GK islets, high glucose failed to increase inositol phosphate accumulation (16). The inability of glucose to stimulate inositol phosphate production is not related to defective phospholipase C activity per se (total activity in islet homogenates is normal). Rather, it is linked to abnormal targeting of the phosphorylation of phosphoinositides; the activity of phosphatidylinositol kinase, which is the first of the two phosphorylating activities responsible for the generation of phosphatidylinositol biphosphate, is clearly reduced (16). Moreover, deficient calcium handling and ATP supply in response to glucose probably also contribute to abnormal activation of phosphatidylinositol kinases and phospholipase C.

Concerning cAMP, it is remarkable that its intracellular content is already very high in GK β-cells at low glucose (17). This is related to increased expression of the cyclase isoforms 2, 3, and 7, and of the Goolf form of Gs proteins (18). Furthermore, cAMP is not further enhanced at increasing glucose concentrations (at variance with the situation in normal β-cells) (17). This suggests that there exists a block in the steps linking glucose metabolism to activation of adenylate cyclase in the GK β-cell. This contrasts strikingly with the capacity of the GK β-cell to respond to glucagon-like protein (GLP)-1 such that it is able to restore the secretory competence to glucose with a clear biphasic response (17). This proves that the glucose-incompetence of the GK β-cell is not irreversible and emphasizes the usefulness of GLP-1 as a therapeutic agent in type 2 diabetes.

β-CELL POPULATION AND β-CELL FUNCTION IN THE GK/Par RAT DURING THE PREDIABETIC PERIOD

Extensive follow-up of the animals after delivery has revealed that GK/Par pups become overtly hyperglycemic between 3 and 4 weeks of age. In GK neonates, total β-cell mass is clearly decreased compared with that in Wistar rats.
such β-cell growth retardation cannot be ascribed to decreased β-cell replication or to increased apoptosis (Fig. 5). We therefore postulate that the recruitment of new β-cells from the precursor pool is defective in the young GK rat; other data from our group (19–23) suggest that the permanently reduced β-cell mass in the GK model reflects a limitation of β-cell neogenesis during early fetal life.

At the same age, under in vitro static incubation conditions, GK rat islets release less insulin at basal glucose; however, they amplify their secretory response to high glucose, leucine, or leucine plus glucose to the same extent as Wistar rat islets (Fig. 6). Therefore, there does not seem to exist a major intrinsic secretory defect in the prediabetic GK rat β-cell. Consequently, the lack of β-cell reactivity to glucose, as seen during the adult period, when the GK rats are overtly diabetic, represents an acquired defect (glucotoxicity?)

In conclusion, the earliest alteration so far detected in the GK/Par rat targets the size of the diabetic, represents an acquired defect (glucotoxicity?).

REFERENCES