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The GK rat beta-cell: A prototype for the diseased human beta-cell in type 2 diabetes?

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ABSTRACT

Increasing evidence indicates that decreased functional beta-cell mass is the hallmark of type 2 diabetes (T2D) mellitus. Nowadays, the debate focuses on the possible mechanisms responsible for abnormal islet microenvironment, decreased beta-cell number, impaired beta-cell function, and their multifactorial aetiologies. This review is aimed to illustrate to what extend the Goto–Kakizaki rat, one of the best characterized animal models of spontaneous T2D, has proved be a valuable tool offering sufficient commonalities to study these aspects. We propose that the defective beta-cell mass and function in the GK model reflect the complex interactions of multiple pathogenic players: (i) several independent loci containing genes responsible for some diabetic traits (but not decreased beta-cell mass); (ii) gestational metabolic impairment inducing an epigenetic programming of the pancreas (decreased beta-cell neogenesis and/or proliferation) which is transmitted to the next generation; and (iii) loss of beta-cell differentiation due to chronic exposure to hyperglycemia/hyperlipidemia, inflammatory mediators, oxidative stress and to perturbed islet microarchitecture.

Type 2 diabetes (T2D) arises when the endocrine pancreas fails to secrete sufficient insulin to cope with the metabolic demand (DeFronzo, 1988; Donath and Halban, 2004; Weir and Bonner-Weir, 2004; Rhodes, 2005), because of beta-cell secretory dysfunction and/or decreased beta-cell mass. Hazard of invasive sampling and lack of suitable non-invasive methods to evaluate beta-cell mass and beta-cell functions are strong limitations for studies of the living pancreas in human. In such a perspective, appropriate rodent models are essential tools for identification of the mechanisms that increase the risk of abnormal beta-cell mass/function and of T2D. Some answers to these major questions are available from studies using the endocrine pancreas of the Goto–Kakizaki (GK) rat model of T2D. It is the aim of the present paper to review the common features that makes studies of the GK beta-cell so compelling.

1. The Goto–Kakizaki Wistar (GK) rat, a valuable model of spontaneous T2D

The GK line was established by repeated inbreeding from Wistar (W) rats selected at the upper limit of normal distribution for glucose tolerance (Goto et al., 1975, 1988; Portha et al., 2001, 2007; Östenson, 2001; Portha, 2005).

Until the end of the 1980s, GK rats were bred only in Sendai (Goto et al., 1975). Colonies were then initiated with breeding pairs from Japan, in Paris, France (Portha et al., 1991), Stockholm, Sweden (Östenson, 2001), Cardiff, UK (Lewis et al., 1996), Coimbra, Portugal (Duarte et al., 2004), Tampa, USA (Villar-Palasi and Farese, 1994). Some other colonies existed for shorter periods during the 1990s in London, UK (Hughes et al., 1994), Aarhus, Denmark, and Seattle, USA (Metz et al., 1999). There are also GK rat colonies derived from Paris in Oxford, UK (Wallis et al., 2004) and Brussels, Belgium (Sener et al., 2001). Also, GK rats are available commercially from the Japanese breeders Charles River Japan (Yokohama), Oriental Yeast (Tokyo), Clea (Osaka), and Takeda Lab Ltd. (Osaka), and from Taconic, USA (www.taconic.com).

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In our colony (GK/Par subline) maintained since 1989, the adult GK/Par body weight is 10–30% lower than that of age- and sex-matched control animals. In male GK/Par rats, non-fasting plasma glucose levels are typically 10–14 mM (6–8 mM in age-matched Wistar (W) outbred controls). Despite the fact that GK rats in the various colonies bred in Japan and outside over 20 years have maintained rather stable degree of glucose intolerance, other characteristics such as beta-cell number, insulin content and islet metabolism and secretion have been reported to differ between some of the different colonies, suggesting that different local breeding environment and/or newly introduced genetic changes account for contrasting phenotypic properties.

Presently it is not clear whether the reported differences are artefactual or true. Careful and extensive identification of GK phenotype within each local subline is therefore necessary when comparing data from different GK sources (Table 1). As an illustration of this point, we have recently compared insulin and glucagon release by GK rats obtained from Taconic (GK/Tac) to that of GK/Par rats, using the perfused pancreas technique (Fig. 1). Despite no significant difference as far as body weight, basal postabsortive plasma glucose level and glucose tolerance to i.v. glucose in vivo are concerned (8 weeks old males), a milder impairment of insulin release in response to glucose (preservation of first phase) or arginine, together with an increased glucagons release in response to arginine, were found in the GK/Taconic as compared to the GK/Par rats. For further details concerning the pathogenic sequence culminating in the chronic hyperglycemia at adult age in the GK/Par rat, please refer to recent reviews (Portha et al., 2001; Östenson, 2001; Portha, 2005; Portha et al., 2007).

2. Less beta-cells

2.1. T2D human pancreas

Whether beta-cell mass is decreased in T2D has been controversial for a long time (Maclean and Ogilvie, 1955; Gepts, 1957; Saito et al., 1979; Stefan et al., 1982; Rahier et al., 1983; Kloppel et al., 1985; Clark et al., 1988; Sempoux et al., 2001; Sakuraba et al., 2002). These discrepancies are in part due to the paucity of available data in humans. It is difficult to obtain pancreatic tissue from humans, since this usually only becomes available at autopsy, when the pancreas may have undergone autolysis. Also, reliable clinical information about autopsy cases is often unavailable. It is therefore not surprising that there are few studies of islet morphology in humans with diabetes, and in most of these, only small numbers of cases were included. However, it was suggested a long time ago that beta-cell mass is in fact reduced in T2D (Hellerström, 1984; Kloppel et al., 1985). Indeed several recent studies utilizing post-mortem and surgical pancreas specimens have provided valuable information on the pathogenesis of islet beta-cell failure in T2D (Guiot et al., 2001; Sakuraba et al., 2002; Butler et al., 2003; Yoon et al., 2003; Deng et al., 2004; Anello et al., 2005) and all of them have shown a reduction in beta-cell mass in T2D. Among these is a landmark study of beta-cell volume together with markers of beta-cell proliferation and apoptosis, taking advantage of the unique autopsy material (124 human pancreases) available at the Mayo Clinic to study from cases with and without diabetes and matched for obesity (Butler et al., 2003). The study showed relative beta-cell volume to be increased by 50% in obese compared with lean nondiabetic pancreas and was attributed to increased neogenesis of islets from exocrine ductal tissue. IGT obese and T2D obese subjects had a 40% and 63% beta-cell volume deficit respectively, compared with weight-matched controls. Lean T2D subjects had a 41% deficit of beta-cell volume compared with lean controls. Furthermore, the decreased volume was not a consequence of reduced beta-cell proliferation, but was associated with increased beta-cell death by apoptosis (Butler et al., 2003). This last finding was confirmed in an independent study, with increased activities of caspase-3 and caspase-8 (Marchetti et al., 2004). Interestingly, these last authors reported that metformin was found to exert an anti-apoptotic effect on the human diabetic beta-cells in vitro, which was paralleled by a reduction of caspase-3 and caspase-8 activities (Marchetti et al., 2004). It is important to remark that, even if accelerated, the rate of beta-cell apoptosis in T2D rarely progresses to near-complete loss, even after decades of the disease. Although it is often assumed that accelerated apoptosis of beta-cells is important for the pathogenesis of T2D, limitations in beta-cell replication and/or neogenesis could be just as important. It is remarkable that despite the decreased total pancreatic beta-cell mass, the percentage of beta-cells within isolated islets was only slightly reduced (by 10%) in T2D patients as compared with controls (Del Guerra et al., 2005). Moreover, the fact that loss of beta-cell mass was evident in subjects with IGT (Butler et al., 2003) suggests that beta-cell mass changes are not necessarily confined to late-stage T2D. Some glucagon or insulin positive cells were observed in the pancreatic ducts in T2D pancreases (Yoon et al., 2003). Beta-cell neogenesis might be increased in T2D because larger numbers of duct cells were found to be insulin immunoreactive in T2D than in normal controls (Jones and Clark, 2001). These findings support alpha and beta-cell neogenesis from precursor duct cells in adult T2D patients, in the face of a low beta-cell replication rate (Yoon et al., 2003).

Most of these studies in human pancreatic tissues suffer from methodological limitations: (1) the relative beta-cell volume is generally used as a surrogate of beta-cell mass. As total pancreas weight is not available, this approach will be in error to the extent that there were differences in the overall mean pancreatic weight among groups; (2) it is difficult to distinguish between the two mechanisms, cell formation and cell death, in human tissue sections mainly because dead cells are removed rapidly from the islet by macrophages and neighboring cells, making it hard to quantify cell death; (3) although cell proliferation can be quantified in tissue sections using markers, this only provides a single snapshot in time; (4) these studies cannot supply prospective informations, given that they were done at autopsy. The beta-cell mass in the patients before onset of the disease is unknown. Thus individuals susceptible to diabetes possibly have limited beta-cell mass early in life, may be even in utero, due to genetic or environmental factors. If such were the case, the decrease in beta-cell mass as seen once T2D is there, would not necessarily be the consequence of the disease itself.

2.2. GK rat pancreas

In the adult hyperglycemic GK/Par rats (males or females), total pancreatic beta-cell mass is decreased (by 60%) (Portha et al., 2001; Movassat et al., 1997). This alteration of the beta-cell population cannot be ascribed to increased beta-cell apoptosis but is related, at least partly, to significantly decreased beta-cell replication as measured in vivo (Portha et al., 2001). The islets isolated by standard collagenase procedure from adult GK/Par pancreases, show limited decreased beta-cell number (by 15% only) and low insulin content compared with control islets (Giroix et al., 1999). The islet DNA content was decreased to a similar extent, consistent with our morphometric data, which indicate that there is no major change in the relative contribution of beta-cells to total endocrine cells in the GK islets. In addition, the insulin content, when expressed relative to DNA, remains lower in GK islets than in control (inbred W/Par) islets, which supports a very limited degranulation in the beta-cells of diabetic animals (Giroix et al., 1999). The distribution of various

Table 1
Similarities and discrepancies of the pancreatic islet/beta-cell phenotypes in the different GK rat sublines so far reported in the literature (March 2008)

Pancreas/islet/beta-cell phenotype	GK/Par	GK/Jap	GK/Sto	GK/UK	GK/Dal	GK/Sea	GK/Coi	GK/Tac
Reduction in beta-cell mass compared with age-/weight-matched controls	√	√	No	?	?	?	?	?
Normal beta-cell apoptosis	√	√	No	?	?	?	?	?
Reduced beta-cell proliferation	√	√	√	?	?	?	?	?
Decreased beta-cell neogenesis	√	?	?	?	?	?	?	?
Slightly decreased alpha-cell proportion within the islets	√	?	No	?	?	?	?	?
Perturbed microenvironment within and around the islets	√	√	?	?	?	?	?	?
Fibrosis, arteriosclerosis, fat infiltration	√	√	?	?	?	?	?	?
Macrophage infiltration of the islets	√	?	?	?	?	?	?	?
Multiple beta-cell functional defects	√	√	√	√	√	√	√	?
Maintenance of active proinsulin gene transcription and translation	√	?	√	?	?	?	?	?
Normal proinsulin biosynthesis and processing	√	√	√	√	?	?	?	?
Slight decrease of beta-cell insulin content	√	√	√	√	√	No	√	?
Markedly reduced activity of the insulin release mechanism, especially in response to glucose	√	√	√	√	√	√	√	√
Reduced GLUT2 expression/glucose uptake	No	No	No	No	√	?	?	?
Reduced glucokinase expression/glucose phosphorylation	No	No	No	?	?	?	?	?
Decreased islet glucose oxidation/glycolysis ratio	√	√	√/no	√	?	?	?	?
Reduced FAD-glycerophosphate dehydrogenase activity	√	√	√	?	?	?	?	?
Reduced glucose-induced mitochondrial membrane hyperpolarization	√	√	√	?	?	?	?	?
Lower ATP/ADP ratio in response to glucose	√	?	√/no	√	?	No	?	?
Impaired islet calcium metabolism	√	√	√	√	?	?	?	?
Decreased activation of islet PI kinases and phospholipase C by glucose	√	?	?	?	?	√	?	?
increase basal islet cAMP level, in relation to increased expression of AC1, AC2, AC3, GαS, Gαolf	√	?	√	?	?	?	?	?
Increased expression of UCP-2	√	?	√	?	?	√	?	?
Increased islet levels of nitrotyrosine and 8-hydroxy-2-deoxyguanosine	?	√	?	?	?	?	?	?
Increased expression of manganese-superoxide and Cu/Zn superoxide dismutases	√	?	?	?	?	?	?	?
Increased expression of catalase and glutathione peroxidase	√	?	?	?	?	?	?	?
Reduced expression of islet SNAREs and SNARE-modulating proteins: synaptotagmin, VAMP-2, syntaxin-1A, SNAP-25, nSec1 and Munc 13-1	√	√	√	?	?	?	?	?
Increased actin expression	No	√	√	?	?	?	?	?
Beta-cell responsiveness to sulfonylureas and GLP-1	√	√	√	√	?	√	?	?
Polygenic aetiology	√	?	√	?	?	?	?	?
Maternal hyperglycemia in pregnancy is an environmental risk factor for diabetes	√	?	?	?	?	?	?	?

GK islet cell types appears to differ between some of the GK rat colonies. Thus, in the Stockholm colony (GK/Sto) beta-cell density and relative volume of insular cells were alike in adult GK rat and control W rats (Östenson, 2001; Abdel-Halim et al., 1993; Guenifi et al., 1995). Similar results were reported in a GK colony in Dallas (GK/Dal) (Ohneda et al., 1993).

A meaningful set of data from our group (Movassat and Portha, 1999; Miralles and Portha, 2001; Plachot et al., 2001; Calderari et al., 2007) suggests that the permanently reduced beta-cell mass in the GK/Par model indeed reflects a limitation of beta-cell neogenesis during early fetal life and thereafter. Follow-up of the animals after delivery revealed that GK/Par pups become overtly hyperglycemic for the first time after 3–4 weeks of age only (i.e. during the weaning period). Despite normoglycemia, total beta-cell mass was clearly decreased (by 60%) in the GK pups when compared with age-related W pups (Movassat et al., 1997). Since this early beta-cell growth retardation in the prediabetic GK/Par rat pups cannot be ascribed to decreased beta-cell replication, nor to increased apoptosis (Movassat et al., 1997), we postulated that the recruitment of new beta-cells from the precursor pool (beta-cell neogenesis) was defective in the young prediabetic GK/Par rat. A comparative study of the development of GK/Par and W pancreases indicates that the beta-cell deficit (reduced by more than 50%) starts as early as fetal age 16 days (E16) (Miralles and Portha, 2001). During the time-window E16–E20, we detected an unexpected anomaly of proliferation and apoptosis of undifferentiated ductal cells in the GK pancreatic rudiments (Miralles and Portha, 2001; Calderari et al., 2007). Therefore, the decreased cell prolif-

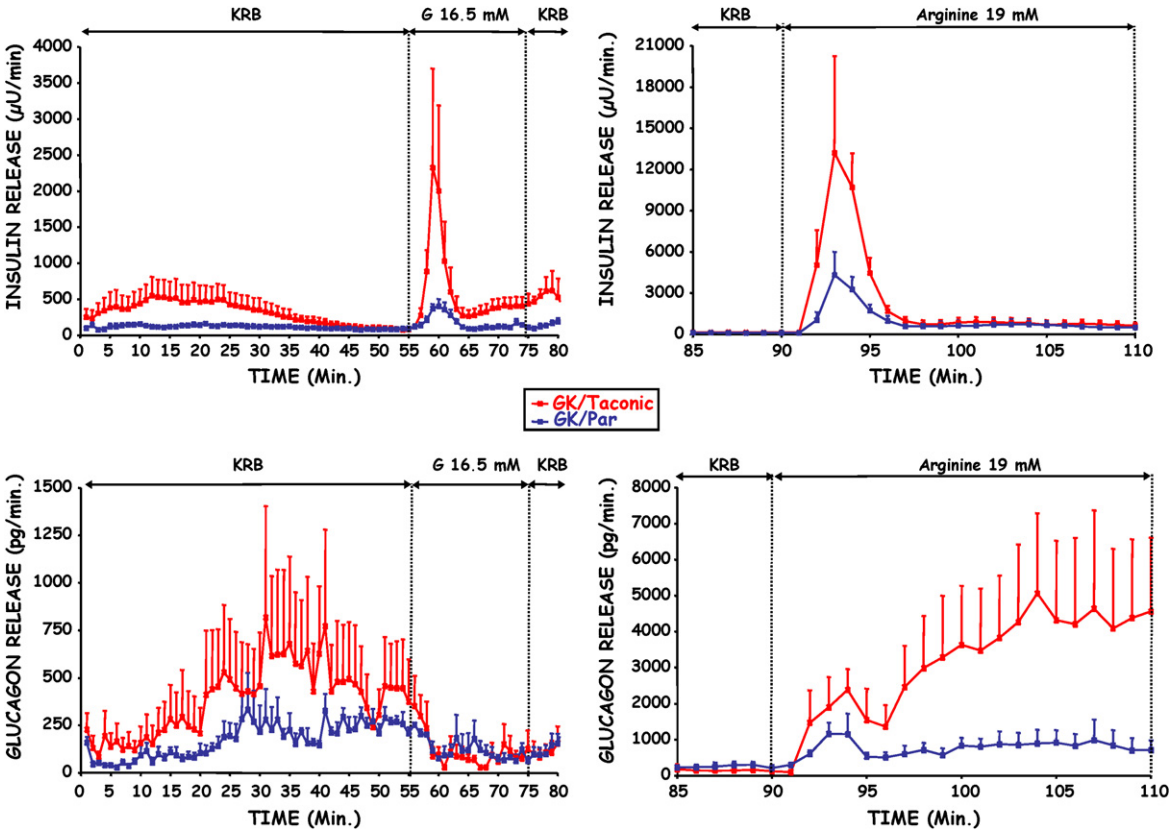

Fig. 1. Insulin (upper panels) and glucagon release (lower panels) in GK/Par (blue symbols, $n = 5$ and GK/Tac (from commercial source, Taconic) (red symbols, $n = 6$) male rat pancreases (isolated perfused pancreases) in the presence of 16.7 mM glucose or 19 mM arginine. When not otherwise indicated, the perfusion medium (KRB) contained no glucose. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

eration and survival in the ductal compartment of the pancreas where the putative endocrine precursor cells localize, suggests that the impaired development of the beta-cell in the GK fetus could result from the failure of the proliferative and survival capacities of the endocrine precursor cells. Importantly, data from our group indicate that defective signaling through the Igf2/Igf1-R pathway represents a primary anomaly since Igf2 and Igf1-R protein expressions are already decreased within the GK pancreatic rudiment at E13.5, at a time when beta-cell mass (first wave of beta-cell expansion) is in fact normal (Calderari et al., 2007). Low levels of pancreatic Igf2 associated with beta-cell mass deficiency are maintained thereafter within the fetal pancreas (Serradas et al., 2002). We have also published data illustrating a poor proliferation and/or survival of the endocrine precursors also during neonatal and adult life (Movassat et al., 1999; Plachot et al., 2001). All together these arguments support the notion that an impaired capacity of beta-cell neogenesis (either primary in the fetus, or compensatory in the newborn and the adult) results from the permanently decreased pool of endocrine precursors in the GK/Par pancreas (Movassat et al., 2007).

During the last few years, some important informations concerning the determinants (morbid genes vs. environment impact) for basal hyperglycemia and glucose intolerance in the GK model, have been supplied. Hyperglycemia experienced during the fetal and/or early postnatal life may contribute to programming of the endocrine pancreas (Simmons, 2006). Such a scenario also potentially applies to the GK/Par model, as GK/Par mothers are slightly hyperglycemic through their gestation and during the suckling period (Serradas et al., 1998). Concerning the potential maternal influence on the development of T2D in the GK model, Gauguier et al. (1994) reported that adult offspring of GK/Par females crossed with W males have a more marked hyperglycemia than adult offspring of W females crossed with GK males, suggesting higher maternal inheritance. However, this conclusion was not confirmed in other studies (Abdel-Halim et al., 1994; Gill-Randall et al., 2004) and cross-breeding experiments do not overcome the difficulty to isolate the respective contribution of genetic vs. intrauterine environmental factors. Recently, Gill-Randall et al. (2004) have used a rat embryo transfer technique to examine more convincingly that major point. First, these authors showed that offspring from GK embryos transferred in the uterus of euglycemic W mother still develop basal hyperglycemia and postprandial glucose intolerance when adults, therefore highlighting a predominant role for genetic factors (Gill-Randall et al., 2004). Second, they also showed that exposure of the W embryo to hyperglycemia in utero (as seen in the GK pregnant mother) increases the risk of hyperglycemia in adult W life (Gill-Randall et al., 2004), this clearly illustrating the notion that there exists indeed a diabetogenic role for the GK intrauterine environment. The Randall's study however did not address to insulin secretion and beta-cell mass. We have preliminary unpublished data using the same experimental embryo-transfer strategy, suggesting that GK embryos transferred in the uterus of euglycemic W mother still develop deficiency of pancreatic insulin stores and beta-cell mass when adults, to the same extend as the GK rats from our stock colony. While this preliminary conclusion rather favours a major role for inheritance of morbid genes, additional studies are needed to really eliminate the option that the gestational diabetic pattern of the GK/Par mothers does not contribute to establish and/or maintain the transmission of endocrine pancreas programming from one GK/Par generation to the next one.

Studies on the offspring in crosses between GK/Par and W rats demonstrated that F1 hybrid fetuses, regardless of whether the mother was a GK or a W rat, exhibit decreased beta-mass and glucose-induced insulin secretion closely resembling those in GK/GK fetuses (Serradas et al., 1998). This finding indicates that conjunction of GK genes from both parents is not required for defective beta-cell mass to be fully expressed. We have also shown that to have one GK parent is a risk factor for a low beta-cell mass phenotype in young adults, even when the other parent is a normal W rat (Calderari et al., 2006).

Two functional point mutations in the promoter region of the adenyl cyclase type 3 (AC3) gene have been reported in both islets and peripheral blood of GK/Sto rats and are associated to beta-cell AC3 over expression and increased cAMP generation (Abdel-Halim et al., 1998). The contribution if any, of such a mutation to the GK beta-cell growth defect is so far unknown.

Gauguier et al. (1996) using a quantitative trait locus (QTL) approach, have identified six independently segregating loci containing genes regulating fasting plasma glucose and insulin levels, glucose tolerance, insulin secretion and adiposity in GK/Par rats. The same conclusion was drawn by Galli et al. (1996) using GK/Sto. This established the polygenic inheritance of diabetes-related parameters in the GK rats whatever their origin. Both studies found the strongest evidence of linkage between glucose tolerance and markers spanning a region on rat chromosome 1, called Niddm1 locus. The Niddm1i locus, located at the telomeric end of chromosome 1 in the GK rat, is also a T2D susceptibility locus in humans and corresponds to human chromosome 10q24–q26 (Duggirala et al., 1999; Grant et al., 2006). While it must be recognized that many of the glucose-controlling locus variants reported in the GK/Par and GK/Sto rats (Gauguier et al., 1996; Galli et al., 1996; Wallis et al., 2004) were associated in fact with hyperinsulinemia or enhanced insulin secretion (and paradoxically not with the reverse), more recent works using congenic technology have identified on the Niddm1i locus a 3.5 cM region containing approximately ten genes, as a major susceptibility locus for defective insulin secretion (Lin et al., 2001). It has been recently reported that beta-cell mass is intact in Niddm1i subcongenics (Granhall et al., 2006). Finally, no QTL association with beta-cell mass or beta-cell size could be found in the GK/Par rat (Ktorza and Gauguier, personal communication of unpublished data). Therefore, the likelihood that a genotype alteration contributes to the low beta-cell mass phenotype in the GK/Par rat is reduced. The raised question to be answered now is whether or not epigenetic perturbation of gene expression occurs in the developing GK pancreas and contributes to the alteration of early beta-cell growth. Igf2 and Igf1r genes are good candidates for such a perspective.

3. A perturbed beta-cell microenvironment within and around the islets

3.1. T2D human islets

Many changes in the islet architecture of T2D subjects (Wittingen and Frey, 1974; Orci et al., 1975; Saito et al., 1979; Baetens et al., 1979; Yoon et al., 2003) have been described including fibrosis, deposition of hyalin-like material replacing beta-cells, abnormal beta-cell distribution within islets, reduced size and number of islets (Yoon et al., 2003; Butler et al., 2003), arteriosclerosis, and fat infiltrations (Butler et al., 2003; Zhao et al., 2003). A prominent finding in the diabetic patients was increased alpha-cell proportions within the islets in some (Yoon et al., 2003; Deng et al., 2004) but not all (Del Guerra et al., 2005) studies, and remarkable heterogeneity of morphological changes of islets within the same lobe of the pancreas (Yoon et al., 2003). This is not correlated to significant alteration of glucagon release since measurement of glucagon secretion by perifused T2D islets did not reveal marked differences versus control preparations (Deng et al., 2004).

One of the features also present in islets of most humans with T2D is amyloid deposits (Clark et al., 1988; O'Brien et al., 1994; Saito et al., 1979; Westermark and Wilander, 1978; Sempoux et al., 2001; Butler et al., 2003). The role of islet amyloid in the islet pathogenesis in T2D remains controversial. One argument that has been made against the pathogenic importance of both islet amyloid in T2D and cerebral amyloid in Alzheimer's disease is that not all affected cases have amyloid present (Butler et al., 2003). It is possible that the formation of islet amyloid is secondary to hyperglycemia and not of primary importance in the pathophysiology of T2D.

Since low-grade chronic inflammation has received increasing attention in recent years as an important pathophysiological mechanism in adipocyte insulin-resistance and atherosclerosis, inflammatory phenomena may also target the islet in T2D. Very recent data indeed support such a view since increased numbers of immune cells, specifically macrophages, were found associated with pancreatic islets in T2D (Ehses et al., 2007a). Increased islet-derived inflammatory factors such as interleukins (IL6, IL8), granulocyte colony-stimulating factor (GCSF) and macrophage inflammatory protein (MIP1alpha) are produced and released when human islet are exposed to a T2D milieu (Ehses et al., 2007a). Further, this inflammatory response was found to be biologically functional as conditioned medium from human islets exposed to a T2D milieu could induce increased migration of monocytes and neutrophils. This migration was blocked by IL8 neutralization and IL8 was localized to human alpha-cells (Ehses et al., 2007a). Thus, islet-derived inflammatory factors are regulated by the T2D milieu and may contribute to the macrophage infiltration of the human islets. Whether the presence of macrophages is causative to T2D islet pathology requires further investigation. Possibly, early infiltration of macrophages may be beneficial to islet function and plasticity. However, as the disease progresses, macrophages may play a role in accelerating pancreatic islet cell dysfunction and death. Macrophages also may be present after beta-cell death, acting to phagocytose dead islet tissue.

3.2. GK rat islets

The adult GK/Par pancreas exhibits two different populations of islets in situ: large islets with pronounced fibrosis (Portha et al., 2001) and heterogeneity in the staining of their beta-cells, and small islets with heavily stained beta-cells and normal architecture. One striking morphologic feature of GK rat islets is the occurrence of these big islets characterized by connective tissue separating strands of endocrine cells (Goto et al., 1988; Suzuki et al., 1992; Guenifi et al., 1995). Accordingly, the mantle of glucagon and somatostatin cells is disrupted and these cells are found intermingled between beta-cells. These changes increase in prevalence with ageing (Suzuki et al., 1992).

Chronic inflammation at the level of the GK/Par islet has recently received demonstration and it is now considered as a pathophysiological contributor in GK diabetes (Ehses et al., 2007a, 2007b). Using an Affymetrix microarray approach to evaluate islet gene expression in freshly isolated adult GK/Par islets, we found that 34% of the 71 genes found to be overexpressed, belong to inflammatory/immune response gene family, and 24% belong to extracellular matrix (ECM)/cell adhesion gene family (Homo-Delarche et al., 2006). Numerous macrophages ($CD68^+$ and MHC class II^+) and granulocytes were found in/around adult GK/Par islets (Homo-Delarche et al., 2006). Up-regulation of the MHC class II gene was also reported in a recent study of global expression profiling in GK

islets from commercial source (Taconic) (Ghanaat-Pour et al., 2007). Immunolocalization with anti-fibronectin and anti-vWF antibodies indicated that ECM deposition progresses from intra- and peri-islet vessels, as it happens in microangiopathy (Homo-Delarche et al., 2006). These data demonstrate that a marked inflammatory reaction accompanies GK/Par islet fibrosis and suggest that islet alterations develop in a way reminiscent of microangiopathy (Ehses et al., 2007b). The previous reports by our group and others that increased blood flow and altered vascularisation are present in the GK/Par and GK/Sto models (Atef et al., 1994; Svensson et al., 1994, 2000) are consistent with such a view. The increased islet blood flow in GK rats may be accounted for by an altered vagal nerve regulation mediated by nitric oxide, since vagotomy as well as inhibition of NO synthase normalized GK/Sto islet flow (Svensson et al., 1994). In addition, islet capillary pressure was increased in GK/Sto rats (Carlsson et al., 1997), this defect was restored after 2 weeks of normalization of glycemia by phlorizin treatment. However the precise relationship between islet microcirculation and beta-cell secretory function remains to be established.

4. Multiple beta-cell functional defects

4.1. T2D human islets

The insulin release defects of T2D patients have been extensively studied in vivo and are mainly characterized by impairment of glucose-stimulated insulin release. Commonly found alterations of insulin secretion in T2D patients include reduced or absent first-phase response to intravenous glucose (Kahn, 2001), delayed or blunted release after ingestion of mixed meals (Polonsky et al., 1998), alterations in rapid pulses and ultradian oscillations (Schmitz et al., 2002). In addition, second-phase insulin secretion and response to nonglucose stimuli may also be reduced in vivo (Weyer et al., 1999; Weir and Bonner-Weir, 2004). More direct assessment of the properties of the diabetic beta-cell using isolated islets is needed to better look for alterations associated with and/or responsible for impaired insulin secretion in T2D. So far, informations related to the functional characteristics of islets from T2D patients are scarce but relatively convergent. Several groups (Fernandez-Alvarez et al., 1994; Lin et al., 2002; Deng et al., 2004; Marchetti et al., 2004; Del Guerra et al., 2005) have recently reported multiple abnormalities of insulin secretion in islets isolated from T2D donors.

The secretion defect in T2D is probably more severe than could be accounted for solely by the reduction in beta-cell mass (Ahren, 2005). The demonstration that human beta-cells maintain active insulin gene transcription and translation even in amyloid-containing islets and that insulin cleavage is normal in most beta-cells (Sempoux et al., 2001), suggests that the major beta-cell functional problem in T2D is abnormal coupling of insulin secretion. Because islet insulin content was only slightly decreased, by <30%, in the islets of T2D patients (Ostenson et al., 2006), it is not likely that the mechanism behind the impaired insulin secretion involves deficient insulin stores but rather reduced activity of the release mechanism.

Beta-cells in T2D showed modest signs of ER stress when studied in pancreatic samples or isolated islets maintained at physiological glucose concentration. However, exposure to increased glucose levels induced ER stress markers in T2D islet cells, which therefore may be more susceptible to ER stress induced by metabolic perturbations (Marchetti et al., 2007). Accordingly, it has been proposed that factors others than ER stress may play a prominent role in beta-cell dysfunction and death, or that beta-cells in T2D face a condition of ER stress that is relatively compensated for periods of time, but exacerbates in case of metabolic decompensation, thus contributing to beta-cell damage (Marchetti et al., 2007).

In islets isolated from the pancreas of multiorgan donors who were affected by T2D, it has been observed a clearly reduced insulin release in response to glucose in vitro, whereas the secretion in response to leucine, glutamine or the non-fuel secretagogue arginine, was only slightly affected (Deng et al., 2004; Marchetti et al., 2004; Del Guerra et al., 2005). The pulsatile nature of glucose-induced insulin release was preserved in T2D islets, but the amplitude of the pulses was reduced (Lin et al., 2002). Glucose stimulation normally results in the transfer of reducing equivalents to the respiratory chain, leading to hyperpolarization of the mitochondrial membrane and generation of ATP. The expression of the SLC2A2/GLUT2 gene was repeatedly reported reduced in islets from T2D patients (Gunton et al., 2005; Ostenson et al., 2006; Del Guerra et al., 2005), while the islet glucokinase expression was reported normal (Ostenson et al., 2006) or reduced (Gunton et al., 2005; Del Guerra et al., 2005). In addition, the activity of FAD-glycerophosphate dehydrogenase, glutamate-oxalacetate transaminase, or glutamate-pyruvate transaminase was lower in T2D islets than in control cells (Fernandez-Alvarez et al., 1994). In T2D islets, glucose-induced activation of glucose oxidation and mitochondrial membrane hyperpolarization was reduced (Del Guerra et al., 2005; Anello et al., 2005); ATP levels were lower at high glucose and the ATP/ADP ratio was blunted, in response to glucose stimulation (Anello et al., 2005). Further support to the concept that mitochondria in the diabetic beta-cell are in an altered state comes from electron microscopy examinations: the density volume of these organelles was significantly higher in T2D beta-cells than in control cells, due to mitochondrial swelling and enlargement (Anello et al., 2005). In the T2D islets, the increased protein expression of complex I and complex V of the respiratory chain (Anello et al., 2005) does not support the possibility for a reduced electron flux through the respiratory chain. Rather the increased expression of UCP-2 (Anello et al., 2005) could be responsible for the reduced hyperpolarization of the mitochondrial membrane, lower ATP levels, ATP/ADP ratio, and eventually, of the reduced insulin release in response to glucose. UCP-2 protein expression could be activated by an increased formation of reactive oxygen species (Krauss et al., 2003).

An association between impaired insulin response to glucose in T2D islets and greatly reduced expression of islet SNARE complex and SNARE-modulating proteins has been recently reported (Ostenson et al., 2006). The secretory vesicle SNAREs, synaptotagmin and VAMP-2, the target membrane-SNAREs, syntaxin-1A and SNAP-25, and the cytosolic SNARE-modulating proteins nSec1 and Munc 13-1, were decreased on either or both mRNA and protein levels. Also actin levels were increased in T2D islets relative to the nondiabetic controls (Ostenson et al., 2006).

Nitrotyrosine derives from the reaction of superoxide and nitric oxide and is considered a reliable marker of oxidative stress. Also 8-hydroxy-2-deoxyguanosine concentration was significantly higher in T2D than control islets, and it was correlated with the degree of glucose-stimulated insulin release impairment (Del Guerra et al., 2005). The concept that the functional defects found in T2D islets are related at least in part to increased oxidative stress, is also consistent with the reports of enhanced oxidative stress-related DNA damage (Sakuraba et al., 2002), increased expression of protein kinase C-beta2 and nicotinamide adenine dinucleotide phosphate reduced-oxidase (Marchetti et al., 2004) and reduced expression of manganese-superoxide and Cu/Zn superoxide dismutases (Sakuraba et al., 2002; Marchetti et al., 2004) in T2D pancreases. On the contrary, the increased expression of catalase and glutathione peroxidase in T2D islets (Marchetti et al., 2004) suggests an attempt to enhance the elimination of ROS through

pathways different from dismutase activity. Accordingly, in vitro 24-h exposure to glutathione (Del Guerra et al., 2005) or metformin (Marchetti et al., 2004), significantly improved glucose-stimulated insulin release and decreased nitrotyrosine concentration in T2D islets. These data provide direct evidence that reducing islet cell oxidative stress is a potential target for therapeutic approaches of the diabetic human beta-cell. They also suggest that the functional decline of T2D islets may not be relentless and can be, at least in part, reversible. This last conclusion is also supported by the observation that the impaired insulin release in islets of T2D patients can be reversed in vitro by GLP-1 (Ostenson et al., 2006). Such a normalization of the insulin response after GLP-1 in T2D patients was demonstrated in previous in vivo studies (Gutniak et al., 1992).

Recently, islets of T2D patients demonstrated marked down-regulation of the aryl hydrocarbon receptor nuclear translocator (ARNT, also called hypoxia-induced factor 1 beta or HIF1β) which regulates a number of genes involved in vascular function and hypoxic response including VEGF, PAI1 and EPO (Gunton et al., 2005).

Fig. 2 illustrates a compendium of the abnormal intracellular sites so far identified in human T2D islets.

The causes underlying the defective insulin secretion in T2D are complex and multiple. It is also liable that one single defect is not sufficient for eliciting a defective function of that degree as diabetes develops, because compensation in other mechanisms may take place. The determinants for beta-cell dysfunction in T2D include genetic, environmental and intrauterine/perinatal factors (Gerich, 1998).

While several rare monogenic forms diabetes (maturity-onset diabetes of the young, MODY), have been described, the genetic basis of T2D is clearly much more complex. T2D is believed to be a polygenic disease in which variations within multiple genes, each adding some risk (Laakso, 2007; Frayling and Mc Carthy, 2007). Through various genetic approaches, polymorphisms within numerous genes relating to pancreatic islet function have been discovered that are associated with decreased/increased risk of T2D. Of particular note are polymorphisms of PPARg, PPARg coactivator 1-a (PGC1a), transcription factor Kruppel-like factor 11 (KLF11), transcription factor 7-like 2 (TCF7L2), calpain 10 (CAP10), KCNJ11 (Kir6.2), and allele III of a variable number tandem repeat 0.5 kb upstream of the insulin genes (INS-VNTR) (Laakso, 2007). For the majority of these polymorphisms, it is uncommon for a significantly increased T2D risk to be shown in more than one or a few population cohorts, consistent with enormous heterogeneity in heritability for this condition. Apparent exceptions are the variants in the transcription factor TCF7L2 and of KCNJ11 (Kir6.2) (Laakso, 2007; Frayling and Mc Carthy, 2007). Interestingly, the increased risk of T2D conferred by variants in TCF7L2 involves the enteroinsular axis, enhanced expression of the gene in islets, and impaired insulin secretion (Lyssenko et al., 2007).

There is also evidence, that maternal hyperglycemia in diabetic pregnancy is also an early life environmental risk factor for T2D (Dabelea and Pettitt, 2001). In other words, the intrauterine metabolic environment may exert remote effects on the adult beta-cell health (metabolic imprinting of the beta-cell) (Simmons, 2006).

4.2. GK rat islets

As for pancreatic beta-cell mass, there is some controversy regarding the content of pancreatic hormones in GK rats. In the adult hyperglycemic GK/Par rats (males), total pancreatic insulin stores are decreased by 60–40%) (Portha et al., 2001). In other GK rat colonies (Stockholm, Japan, Seattle, Tampa), insulin store values have been found similar or more moderately decreased, compared with control rats (Östenson et al., 1993b; Abdel-Halim et al., 1993; Suzuki et al., 1997; Salehi et al., 1999; Metz et al., 1999). No major alteration in pancreatic glucagon content, expressed per pancreatic weight, has been demonstrated in GK/Sto rats (Abdel-Halim et al., 1993), although the total alpha-cell mass was decreased by about 35% in adult GK/Par rats (Movassat et al., 1997). The periph-

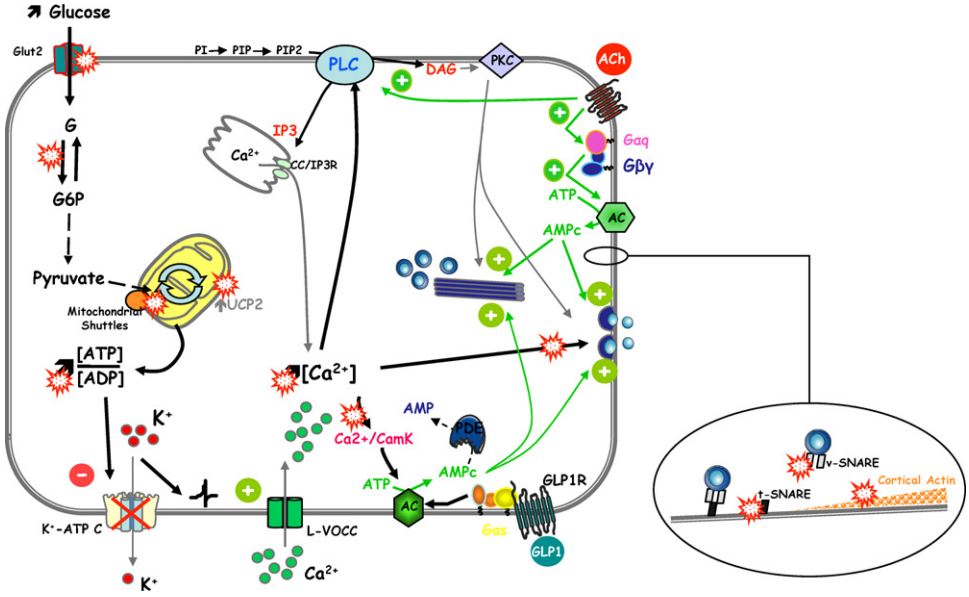

Fig. 2. Model for defective glucose-induced insulin release and the abnormal intracellular sites so far identified in the beta-cells obtained from humans with T2D. Where data are available, the impaired sites in the beta-cell are indicated with the symbol: ✸.
Abbreviations—Glut: glucose transporter; AC, adenylate cyclases; Gαs, Gαq: α subunits of heterotrimeric G proteins; Gβγ: β and γ subunits of heterotrimeric G proteins; PI, PIP, PIP2: phosphoinositides; PLC: phospholipase C; PKC: protein kinase C; DAG: diacylglycerol; IP3: inositol-3-phosphate; UCP-2: uncoupling protein 2; tSNARE, v-SNARE: SNARE proteins (syntaxin-1A, SNAP-25, VAMP-2, Munc 18, Munc 13-1, synaptophysin); L-VOCC: L-type calcium channel modulated by the membrane polarization; CC/IPR3: calcium channel modulated by receptor to IP3; K/ATP C: potassium channel modulated by the ATP/ADP ratio; Ca^{2+}/CamK: calcium calmoduline kinase; Ach: acetylcholine; M3-R: muscarinic receptor isoform 3; GLP-1: glucagon-like peptide 1; GLP-1-R: GLP-1 receptor. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

eral localization of glucagon-positive cells in W islets was replaced in GK/Sto rats with a more random distribution throughout the core of the islets (Guest et al., 2002). Pancreatic somatostatin content was slightly but significantly increased in GK/Sto rats (Abdel-Halim et al., 1993).

Glucose-stimulated insulin biosynthesis in freshly isolated GK/Par, GK/Jap or GK/Sto islets has been reported grossly normal (Giroix et al., 1993b; Nagamatsu 1999; Guest et al., 2002). The rates of biosynthesis, processing and secretion of newly synthesized (pro)insulin were comparable (Guest et al., 2002). This is remarkable in the face of markedly lower prohormone convertase PC2 immunoreactivity and expression in the GK/Sto islets, while the expression patterns of insulin, PC1, PC2, carboxypeptidase E (CPE) remained normal (Guest et al., 2002). Circulating insulin immunoreactivity in GK/Sto rats was predominantly insulin 1 and 2 in the expected normal ratios with no (pro)insulin evident. The finding that proinsulin biosynthesis and processing of proinsulin appeared normal in adult GK rats suggests that the depletion of secreted constituents in beta-cells does not arise from a failure to recognize glucose as an activator of prohormone biosynthesis and granule biogenesis. Rather it points to an inability of the beta-cell population as a whole to meet the demands upon insulin secretion imposed by chronic hyperglycemia in vivo. Although circulating insulin levels did not differ between GK and W rats, they were inappropriate for the level of glycemia, indicative of a secretory defect.

Impaired glucose-stimulated insulin secretion has been repeatedly demonstrated in GK rats (whatever the colony), in vivo (Portha et al., 1991; Gauguier et al., 1994, 1996; Galli et al., 1996; Salehi et al., 1999), in the perfused isolated pancreas (Portha et al., 1991; Östenson et al., 1993a, 1993b; Abdel-Halim et al., 1993, 1994, 1996), and in freshly islolated islets (Östenson et al., 1993a, 1993b; Giroix et al., 1993a, 1993b; Hughes et al., 1994). A number of alterations or defects have been shown in the stimulus-secretion coupling for glucose in GK islets. GLUT2 is underexpressed, but not likely to the extent that it could explain the impairment of insulin release (Ohneda et al., 1993). This assumption is supported by the fact that glucokinase/hexokinase activities are normal in GK rat islets (Östenson et al., 1993a; Tsuura et al., 1993; Giroix et al., 1999). In addition, glycolysis rates in GK rat islets are unchanged or increased compared with control islets (Östenson et al., 1993b; Giroix et al., 1993a, 1993b, 1993c; Hughes et al., 1994, 1998; Ling et al., 1998, 2001). Furthermore, oxidation of glucose has been reported decreased (Giroix et al., 1993b), unchanged (Östenson et al., 1993b; Hughes et al., 1994, 1998; Giroix et al., 1993c), or even enhanced (Ling et al., 1998). Also, lactate dehydrogenase gene expression (this paper, Fig. 3) and lactate production (Ling et al., 1998) are increased and pyruvate dehydrogenase activity is decreased (Zhou et al., 1995) in GK rat islets. In GK/Par islets, we showed that mitochondria of exhibit a specific decrease in the activities of FAD-dependent glycerophosphate dehydrogenase (Giroix et al., 1993b, 1993c) and branched-chain ketoacid dehydrogenase (Giroix et al., 1999). Although this certainly could contribute to lower oxidation rates, it does not exclude other mechanisms. Indeed, we found that the beta-cells of adult GK/Par rats had a significantly smaller mitochondrial volume compared to control beta-cells (Serradas et al., 1995). No major deletion or restriction fragment polymorphism could be detected in mtDNA from adult GK/Par islets (Serradas et al., 1995); however, they contained markedly less mtDNA than in control islets. The lower islet mtDNA was paralleled by decreased content of some islet mt mRNAs such as cytochrome b (Serradas et al., 1995). In accordance with this, insufficient increase of ATP generation in response to high glucose was shown by our group (Giroix et al., 1993c). This supports the hypothesis that the defective insulin response to glucose in GK islet is accounted for by an impaired ATP production, closure of the ATP-regulated K+-channels (Tsuura et al., 1993) and impaired elevation of intracellular [Ca^{2+}] (Hughes et al., 1998; Marie et al., 2001; Dolz et al., 2005). Such a view validated in the GK/Par beta-cell, is however contradictory to the reports in GK/Sto and GK/Sea islets that the rate of ATP production is unimpaired (Ling et al., 1998; Metz et al., 1999). Other energy metabolism defects identified in GK/Sto islets, include increased glucose cycling due to increased glucose-6-phosphatase activity (Östenson et al., 1993b; Ling et al., 1998); impaired glycerol phosphate shuttle due to markedly reduced activity of the FAD-linked glycerol phosphate dehydrogenase (Östenson et al., 1993a; MacDonald et al., 1996), and decreased pyruvate carboxylase activity (MacDonald et al., 1996). It is possible that these alterations may affect ATP concentrations locally. However, the enzyme dysfunctions were restored by normalization of glycemia in GK/Sto rats (MacDonald et al., 1996; Ling et al. unpublished observations), but with only partial improvement of glucose-induced insulin release. Hence, it is likely that these altered enzyme activities result from a glucotoxic effect rather than being primary causes behind the impaired secretion. Also, lipotoxic effects leading to defective insulin release have been observed in GK rats on high-fat diet (Shang et al., 2002; Briaud et al., 2002), possibly mediated by a mechanism partly involving modulation of UCP-2 expression.

Phosphoinositides (Dolz et al., 2005) and cyclic AMP metabolism (Dolz et al., 2005, 2006) are also affected in GK/Par islets. While car-

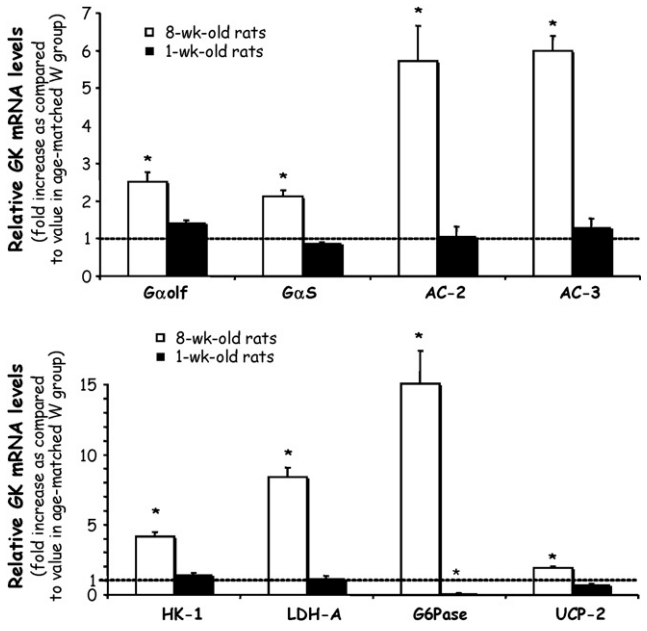

Fig. 3. mRNA expression was altered in islets freshly isolated from adult (8-week-old) diabetic or neonatal (1-week-old) prediabetic GK/Par rats. Quantitative real-time PCR was used to measure expression in islets with age-matched correction for expression of the control S18 gene.

Of the cAMP metabolism genes, adenylyl cyclase isoforms 2 (AC2) and 3 (AC3) together with the α subunits GαS and Gαolf of the heterotrimeric G proteins, were significantly increased in adult GK islets when compared to adult control Wistar islets. By contrast, their expression in neonatal GK rats islets did not differ significantly from the controls. This is consistent with the view that the increased cAMP production by adult GK islets reflects an acquired adaptation possibly ascribed to gluco-lipotoxicity.

Concerning some proteins involved in glucose metabolism pathway or control of mitochondrial oxidative phosphorylation, hexokinase-1 (HK-1), lactate-dehydrogenase A (LDH-A), glucose-6-phosphatase (G6Pase) and uncoupling protein-2 (UCP-2) were significantly increased in adult GK islets when compared to adult control Wistar islets. By contrast, their expression in neonatal GK rats islets did not differ significantly from the controls. $^{*}p < 0.01$ as compared to age-related control group.

bachol was able to promote normal inositol generation in GK/Par islets, high glucose failed to increase inositol-phosphate accumulation (Giroix, unpublished data). The inability of glucose to stimulate IPs production is not related to defective phospholipase C activity per se (total activity in islet homogenates is normal). It is rather linked to abnormal targeting of the phosphorylation of phosphoinositides: the activity of phosphatidyl-inositol kinase, which is the first of the two phosphorylating activities responsible for the generation of phosphatidyl-inositol biphosphate, is clearly reduced (Giroix, unpublished data). Moreover, deficient calcium handling and ATP supply in response to glucose probably also contribute to abnormal activation of PI kinases and phospholipase C.

Concerning cAMP, it is remarkable that its intracellular content is very high in GK/Par beta-cells already at low glucose (Dolz et al., 2005). This is related to increased expression of the adenylyl cyclase isoforms 1, 2 and 3, and of the GαS and Gαolf proteins while the phosphodiesterases PDE3B and PDE1C isoforms are decreased (Dolz et al., 2005, 2006). Furthermore, cAMP is not further enhanced at increasing glucose concentrations (at variance with the situation in normal beta-cells) (Dolz et al., 2005, 2006). This suggests that there exists a block in the steps linking glucose metabolism to activation of adenylate cyclase in the GK/Par beta-cell. An attractive possibility which has been explored in the GK/Sto rat, is that increased AC3 is due to functional mutations in the promoter region of the Ac3 gene (Abdel-Halim et al., 1998). We do not retain this hypothesis in the GK/Par islet since we found that the expression of the AC1, 2 and 3, and of the GαS and Gαolf proteins, are not increased in the prediabetic GK/Par islets (Fig. 3). The constitutively increased cAMP production may enhance the activity of lactate dehydrogenase (Derda et al., 1980). This would in turn lead to increased glucose utilization and reduced cytoplasmic pool of NADH. It is well documented that altered NADH/NAD ratio is associated with impaired glucose-induced insulin release (Eto et al., 1999). The increased cAMP production has also offered the possibility to fully restore the beta-cell secretory competence to glucose in GK/Par as well as GK/Sto islets (Abdel-Halim et al., 1996; Dolz et al., 2006) with a clear biphasic response (Dolz et al., 2006). This also proves that the glucose-incompetence of the GK/Par beta-cell is not irreversible, and emphasizes the usefulness of GLP-1 as a therapeutic agent in T2D. Also, cholinergic stimulation has been demonstrated to restore glucose-induced insulin secretion from GK/Sto as well as GK/Par islets (Guenifi et al., 2001; Dolz et al., 2005). We have proposed that such a stimulation is not mediated through activation of the PKC pathway, but via a paradoxical activation of the cAMP/PKA pathway to enhance Ca^{2+}-stimulated insulin release in the GK/Par beta-cell (Dolz et al., 2005).

Along to these upstream abnormalities, important defects reside late in signal transduction i.e., in the exocytotic machinery. Indeed, glucose-stimulated insulin secretion was markedly impaired in GK/Sto, GK/Jap, GK/Sea and GK/Par islets also when the islets were depolarized by a high concentration of potassium chloride and the ATP-regulated K+-channels kept open by diazoxide (Okamoto et al., 1995; Abdel-Halim et al., 1996; Metz et al., 1999). Similar results were obtained when insulin release was induced by exogenous calcium in electrically permeabilized GK/Jap islets (Okamoto et al., 1995). In fact, markedly reduced expression of several exocytotic SNARE complex proteins, important for the docking and fusion between insulin granules and beta-cell membrane, have been demonstrated in GK/Sto and GK/Jap islets (Nagamatsu et al., 1999; Gaisano et al., 2002; Zhang et al., 2002). Thus, a reduced number of docking granules accounts for impaired beta-cell secretion (Ohara-Imaizumi et al., 2004) and this defect may partly be related to glucotoxicity (Gaisano et al., 2002). Actin cytoskeleton has been implicated also in regulated exocytosis. It has been proposed that in secretory cells, actin network under the plasma membrane acts as a physical barrier preventing the access of secretory granules to the membrane. The level of total actin protein evaluated by western blotting has been found similar in GK/Par and W islets (Movassat, unpublished data), at variance with reports in others GK rat lines (Nagamatsu et al., 1999; Gaisano et al., 2002). However, confocal

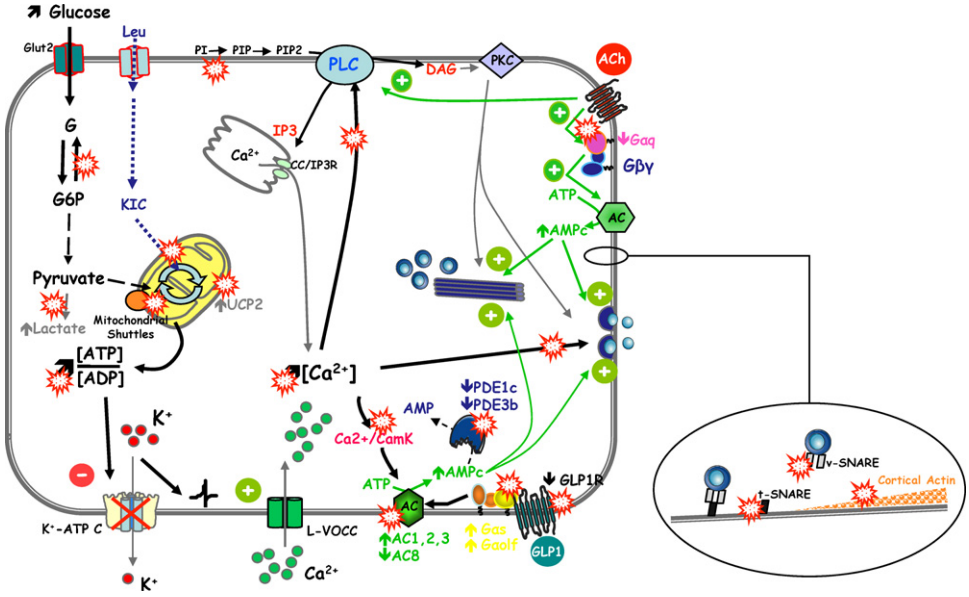

Fig. 4. Model for defective glucose-induced insulin release and the abnormal intracellular sites so far identified in the beta-cell of the diabetic GK rats from the different sources. Where data are available, the impaired sites in the beta-cell are indicated with the symbol: ✸.

Abbreviations—Glut2: glucose transporter isoform 2; Leu: leucine; KIC: keto-isocaproate; AC: adenylate cyclase isoforms; GαS, Gαolf, Gαq: α subunits of heterotrimeric G proteins; Gβγ: β and γ subunits of heterotrimeric G proteins; PI, PIP, PIP2: phosphoinositides; PLC: phospholipase C; PKC: protein kinase C; DAG: diacylglycerol; IP3: inositol-3-phosphate; UCP-2: uncoupling protein 2; tSNARE, v-SNARE: SNARE proteins (syntaxin-1A, SNAP-25, VAMP-2, Munc 18); L-VOCC: L-type calcium channel modulated by the membrane polarization; CC/IPR3: calcium channel modulated by receptor to IP3; K/ATP C: potassium channel modulated by the ATP/ADP ratio; Ca^{2+}/CamK: calcium calmoduline kinase; Ach: acetylcholine; M3-R: muscarinic receptor isoform 3; GLP-1: glucagon-like peptide 1; GLP-1-R: GLP-1 receptor; PDE: cAMP-dependant phosphodiesterase isoforms. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

analysis of the distribution of phalloidin-stained cortical actin filaments revealed a higher density of the cortical actin web nearby the plasma membrane in GK/Par islets as compared to W. Moreover preliminary functional results suggest that the higher density of actin cortical web in the GK/Par islets contribute to the defects in glucose-induced insulin secretion exhibited by GK islets (Movassat, unpublished data).

Other intriguing aspects of possible mechanisms behind defective glucose-induced insulin release in GK/Sto rat islets are the findings of dysfunction of islet lysosomal enzymes (Salehi et al., 1999), as well as marked impairment of the glucose-heme oxygenase-carbon monoxide signaling pathway (Mosén et al., 2005). Diminished levels and/or abnormal activation of several PKC isoenzymes in GK/Sto islets could also account for the defective signals downstream to glucose metabolism, responsible for impaired insulin secretion (Warwar et al., 2006). Peroxovanadium is an inhibitor of islet protein-tyrosine phosphatase (PTP) activities that was shown to enhance glucose-stimulated insulin secretion from GK/Sto islets (Abella et al., 2003; Chen and Östenson, 2005). One possible target for this effect could be PTP sigma that is overexpressed in GK/Sto islets (Östenson et al., 2002). In addition, defects in islet protein histidine phosphorylation have been proposed to contribute to impaired insulin release in GK/Sea islets (Kowluru, 2003).

Considerable interest has recently been focused on the putative role of oxidative stress upon deterioration of beta-cell function/survival in diabetes. T2D in the GK/Jap rats is indeed associated with beta-cell oxidative damages as attested by increased levels 8-OHdG and 4-hydroxy-2-nonenal-modified proteins (Ihara et al., 1999), and treatment of GK/Jap rats with anti-oxydants provided some protection from glucose toxicity (Ihara et al., 2000). Recent data from our group indicate that the GK/Par beta-cell phenotype resulted in a reduced sensitivity to the acute deleterious effect of streptozotocin or H_2O_2 upon the glucose-induced insulin secretion together with an increased expression of the antioxidant gene glutathione peroxydase-1 (Portha et al., 2007). This suggests that an increased expression of antioxidants may confer paradoxically greater ROS scavenging capacity to the GK/Par beta-cell.

Fig. 4 illustrates a compendium of the abnormal intracellular sites so far identified in the diabetic GK islets from the different sources.

There are several arguments indicating that the GK beta-cell secretory failure is, at least partially, related to the abnormal metabolic environment (gluco-lipotoxicity). When studied under in vitro static incubation conditions, islets isolated from normoglycemic (prediabetic) GK/Par pups, amplified their secretory response to high glucose, leucine or leucine plus glutamine to the same extent as age-related W islets (Portha et al., 2001). This suggests that there does not exist a major intrinsic secretory defect in the prediabetic GK/Par beta-cells which can be considered as normally glucose-competent at this stage, at least when tested in vitro. In the GK/Par rat, basal hyperglycemia and normal to very mild hypertriglyceridemia are observed only after weaning (Portha et al., 2001). The onset of a profound alteration in glucose-stimulated

Table 2
Comparison of the endocrine pancreas phenotype in T2D patients and diabetic GK rats: many similarities, few discrepancies

Pancreas/islet/beta-cell phenotype	T2D patient	Diabetic GK/Par rat
Reduction in beta-cell mass compared with age-/weight-matched controls	√	√
Increased beta-cell death by apoptosis	√	No
Reduced beta-cell proliferation	No	√
Decreased beta-cell neogenesis	No	√
Increased alpha-cell proportion within the islets	Yes/no	No
Perturbed microenvironment within and around the islets	√	√
Fibrosis, arteriosclerosis, fat infiltration	√	√
Islet amyloid	√	No
Macrophage infiltration of the islets	√	√
Multiple beta-cell functional defects.	√	√
Maintenance of active proinsulin gene transcription and translation,	√	√
Normal proinsulin biosynthesis and processing	Unknown	√
Slight decrease of beta-cell insulin content	√	√
Very modest signs of beta-cell ER stress	√	Unknown
Markedly reduced activity of the insulin release mechanism, especially in response to glucose	√	√
Reduced GLUT2 expression	√	No
Reduced glucokinase expression	Yes/no	No
Decreased islet glucose oxidation/glycolysis ratio	√	√
Reduced FAD-glycerophosphate dehydrogenase activity	√	√
Reduced glucose-induced mitochondrial membrane hyperpolarization	√	√
Lower ATP/ADP ratio in response to glucose	√	√
Impaired islet calcium metabolism	Unknown	√
Decreased activation of islet PI kinases and phospholipase C by glucose	Unknown	√
increase basal islet cAMP level, in relation to increased expression of AC1, AC2, AC3, GαS, Gαolf	Unknown	√
Increased expression of UCP-2	√	√
Increased islet levels of nitrotyrosine and 8-hydroxy-2-deoxyguanosine	√	√
Reduced expression of manganese-superoxide and Cu/Zn superoxide dismutases	√	No
Increased expression of catalase and glutathione peroxidase	√	√
Reduced expression of islet SNAREs and SNARE-modulating proteins: synaptotagmin, VAMP-2, syntaxin-1A, SNAP-25, nSec1 and Munc 13-1	√	√
Increased actin expression	√	No
Major decrease (90%) in expression of the transcription factor ARNT (HIF1β)	√	Unknown
Beta-cell responsiveness to sulfonylureas and GLP-1	√	√
Polygenic aetiology	√	√
Increased risk of T2D conferred by variants in TCF7L2	√	Unknown
Enhanced islet expression of TCF7L2	√	√
Maternal hyperglycemia in pregnancy is an environmental risk factor for diabetes	√	√

insulin secretion by the GK/Par beta-cell (after weaning) is time-correlated with the exposure to the diabetic milieu. These changes in islet function could be ascribed, at least in part, to a loss of differentiation of beta-cells chronically exposed to even mild chronic hyperglycemia and elevated plasma non-esterified fatty acids. The view that the lack of beta-cell reactivity to glucose as seen during the adult period when the GK rats are hyperglycemic in the basal state, at least partly reflects an acquired defect ascribed to gluco-lipotoxicity, is supported by the reports that chronic treatment of GK rats with phlorizin partially improved glucose-induced insulin release (Nagamatsu et al., 1999; Gaisano et al., 2002; Ling et al., 2001; Portha et al., 2007), while hyperlipidemia induced by high-fat feeding markedly impaired insulin secretion (Briaud et al., 2002).

However, there are now convincing indications in the GK/Sto rat, that two distinct loci encode separately defects in beta-cell glucose metabolism and insulin exocytosis (Granhall et al., 2006). Generation of a series of congenic rat strains harboring different parts of GK/Sto-derived Niddm1i has recently enabled fine mapping of this locus. Congenic strains carrying the GK genotype distally in Niddm1i displayed reduced insulin secretion in response to both glucose and high potassium, as well as decreased single-cell exocytosis. Interestingly, the gene encoding for transcription factor TCF7L2 is also located in this locus and has recently been identified as a candidate gene for T2D in humans (Grant et al., 2006). However, TCF7L2 RNA levels were not different in this GK strain compared with controls (Granhall et al., 2006). By contrast, the strain carrying the GK genotype proximally in Niddm1i exhibited both intact insulin release in response to high potassium and intact single-cell exocytosis, but insulin secretion was suppressed when stimulated by glucose. Islets from this strain also failed to respond to glucose by increasing the cellular ATP-to-ADP ratio. Since the congenics had not developed overt hyperglycemia and their beta-cell mass was found normal, it was concluded their functional defects in glucose metabolism and insulin exocytosis were encoded by two distinct loci within Niddm1i (Granhall et al., 2006).

In conclusion, careful comparison of the alterations so far detected in the T2D human beta-cell population and those found in the diabetic GK beta-cell population (as it is summarized in Table 2), put into the front stage a number of striking commonalities. To the best of our knowledge, none of the rodent models of spontaneous T2D so far available have revealed so close appropriateness for modelling the human diabetic beta-cell. Of course, the GK beta-cell is not a blueprint for the diseased beta-cell in human. There are however sufficient similarities with high value, to justify more efforts to understand the aetiopathogenesis of T2D in this rat model.

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