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Sesiones

From Pathogenesis to Therapeutic of Type 2 Diabetes. The GK Rat Paradigm

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ABSTRACT

Now that the reduction in beta-mass has been clearly established in humans with type 2 diabetes mellitus (T2D), the debate focuses on the possible mechanisms responsible for abnormal islet microenvironment, decreased beta-cell number and impaired beta-cell function, and their multifactorial aetiologies. The informations available in the Goto-Kakizaki (GK/Par line) rat, one of the best characterized animal models of spontaneous T2D, are reviewed in such a perspective. We propose that the defective beta-cell mass and function in the GK/Par model reflects the complex interactions of three pathogenic players: i) several independent loci containing genes responsible for some diabetic traits (but not decreased beta-cell mass); (ii) gestational metabolic impairment inducing a programming of endocrine pancreas (decreased beta-cell neogenesis) which is transmitted to the next generation; and (iii) secondary (acquired) loss of beta-cell differentiation due to chronic exposure to hyperglycaemia (glucotoxicity). An important message is that the «heritable» determinants of T2D do not simply rely on genetic factors, but

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probably involve transgenerational epigenetic responses. Finally, studies from our group have shown that pharmacological use of GLP-1 receptor agonist *in vivo* during the GK prediabetes period, induced beta-cell regeneration through activation of beta-cell replication and neogenesis, and doing so prevented the development of hyperglycaemia. This suggests a novel application of GLP-1 receptor agonists to the prevention of human diabetes by treatment of at risk individuals during the prediabetic period. Since we also demonstrated that GLP-1 acutely restores the glucose competence of the GK beta-cell, GLP-1 receptor agonists turn to be very attractive tools for the treatment of the decreased beta-cell functioning mass as encountered in T2D.

Key words: Type 2 diabetes.—GK rat.—Insulin secretion.—GLP-1R agonists.—Beta-cell regeneration.

RESUMEN

De la patogénesis al tratamiento de la diabetes tipo 2. El paradigma de la rata GK

Ahora que la reducción de la masa de la célula beta ha sido claramente establecida en humanos con diabetes mellitas tipo 2 (T2D), el debate se focaliza sobre los posibles mecanismos responsables de un microambiente anormal en el islote, del decrecido número de células beta, del alterado funcionamiento de ellas y de sus etiologías multifactoriales. Las eficaces informaciones proporcionadas por la rata Goto-Kakizaki (línea GK), uno de los mejor caracterizados modelos animales de T2D espontánea, están siendo revisadas para tales fines. Nosotros proponemos que la defectuosa masa y función de la célula beta en el modelo GK reflejan unas interacciones complejas de tres vertientes patogénicas: i) varios loci que contienen genes responsables de algunos indicios diabéticos (pero no de decrecida masa de célula beta); ii) daño metabólico gestacional que induce un programa de páncreas endocrino (decrecida neogénesis de célula beta), lo cual es transmitido a la próxima generación, y iii) secundaria (adquirida) pérdida de diferenciación de célula beta debida a una exposición crónica a hiperglucemia (glucotoxicidad). Un mensaje importante es que los determinantes «heredables» de T2D no descansan simplemente sobre factores genéticos, sino probablemente envuelven respuestas epigenéticas transgeneracionales.

Finalmente, estudios de nuestro grupo han mostrado que el uso farmacológico de agonistas del receptor de GLP-1 *in vivo*, en el periodo de prediabetes de ratas GK, inducían regeneración de célula beta, a través de la activación de la replicación y neogénesis de célula beta y de esta forma prevenían el desarrollo de hiperglucemia. Esto sugiere una nueva aplicación del receptor agonista de GLP-1 para la prevención de diabetes humana por el tratamiento con éste, durante el periodo prediabético, a individuos de riesgo. Como nosotros mostramos, también, que el GLP-1 restaura de forma acusada la sensibilidad a la glucosa de la célula beta GK, los agonistas del receptor de GLP-1 vuelven a ser una herramienta atractiva para

el tratamiento del decrecimiento de la masa funcionante de la célula beta lo cual es encontrada en T2D.

Palabras clave: Diabetes tipo 2.—Rata GK.—Secreción de insulina.—Agonistas GLP-1R.—Regeneración de células beta.

Diabetes mellitus is a heterogeneous group of disorders characterized by high blood glucose levels. The pancreatic beta-cell and its secretory product insulin are central in the pathophysiology of diabetes (1). Type 1 or insulin-dependent diabetes mellitus results from an absolute deficiency of insulin due to autoimmune beta-cell destruction. In type 2, non-insulin-dependent diabetes mellitus (T2D), liver, muscle and fat cells are resistant to insulin actions and the compensatory attempt by the beta-cell to release more insulin is not sufficient to maintain blood glucose levels within a normal physiological range, finally leading to the functional exhaustion of the surviving beta-cells (1). T2D is made up of multiple forms each of which is characterized to variable degrees by insulin resistance and beta-cell dysfunction, and which together lead to hyperglycemia. At each end of this spectrum are single-gene disorders that affect the ability of the beta-cell to secrete insulin or the ability of liver, muscle and fat cells to respond to insulin's actions.

BETA-CELL DYSFUNCTION AS A CAUSE FOR HUMAN TYPE 2 DIABETES

In patients with recognized type 2 diabetes, abnormalities of secretion are present together with insulin resistance and cause glucose intolerance. So far, information related to the functional characteristics of islets from T2D patients is limited. Several groups (2, 3) have recently reported multiple abnormalities of insulin secretion in islets isolated from T2D donors such as: reduced insulin content, poor secretion in response to glucose (whereas leucine, glutamine or arginine challenge remained effective) associated with a marked alteration of mitochondrial function (diminished glucose oxidation, lower ATP/ADP ratio, impaired hyperpolarization of mitochondrial membrane, increased expression of UCP-2) and signs of increased oxidative stress. Structural changes in the islets of T2D

subjects have been also described including the arteriosclerosis, deposition of amyloid associated fibrosis and fat infiltrations (4, 5).

The genetic basis of beta-cell dysfunction in this form of diabetes (the most frequent one) is certainly more complex than in subjects with MODY or mitochondrial diabetes: it involves both multiple interacting genes and environmental factors, which determine whether diabetes will develop and at what age.

The clustering of T2D in families and the high concordance rates noted for identical vs. fraternal twins implies a genetic etiology. However, after years of intensive work by many laboratories, there has been little success identifying T2D susceptiblity genes in humans (review in 6).

A role for maternal inheritance in T2D was first suggested by epidemiological studies. Adult patients described as having maturity-onset diabetes had a higher prevalence of T2D in the maternal side over two generations compared to the paternal side suggesting a higher maternal transmission (7). In addition, in patients with gestational diabetes, higher frequency of diabetes in mothers than in fathers was reported (8). The maternal influence in the development of T2D has been reported in the majority of studies (9). Although a few studies did not find a maternal effect, none reported a higher paternal transmission. Foetal exposure to T2D as an environmental factor that may explain the maternal transmission of T2D, was first demonstrated in Pima Indians, a population with the highest prevalence of T2D reported around the world (10). In order to negate the confounding effect of genetic factors related to T2D, the effect of in utero exposure to type 1 diabetes was recently studied in a group of adult offspring free from immunological markers of type 1 diabetes: a 33% prevalence of IGT was reported in offspring of mothers compared to none of the offspring of fathers (control group) (11). Taken together, these findings strongly suggest that in utero exposure to diabetes is associated with abnormal glucose homeostasis in offspring of diabetic mothers and may participate in the excess of maternal transmission in T2D.

To sum-up, now that the reduction in beta-mass has been repeatedly established in humans with T2D, the debate focuses on

the possible mechanisms responsible for decreased beta-cell number and impaired beta-cell function and their multifactorial aetiology.

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 Goto-Kakizaki (GK) rat model of T2D and they are reviewed in the present paper. The GK rat is a non obese substrain of Wistar rat origin, developing T2D early in life. Mild fasting hyperglycaemia and postprandial glucose intolerance are primarily due to impaired beta-cell mass and function on the background of a polygenic inheritance. In addition, secondary defects in beta-cell function and insulin action may superimpose (e.g., due to chronic hyperglycaemia [glucotoxicity]). Since the GK rat can be regarded as one of the best available rodent strains for the study of inherited T2D, it is extensively used in preclinical diabetes research.

THE GOTO-KAKIZAKI WISTAR (GK) RAT MODEL OF SPONTANEOUS TYPE 2 DIABETES

Most rodent models used for studies of the inheritance of T2D (ob/ob mouse, db/db mouse, ZDF rat, OLEFT rat) show association of hyperglycaemia and obesity with insulin resistance. However, in the inbred GK (Goto-Kakizaki) rat line and more specifically in our colony (GK/Par subline) maintained since 1989, all rats are nonobese, nonketotic, and display mild fasting hyperglycaemia. The GK line was established by repeated inbreeding from Wistar (W) rats selected at the upper limit of normal distribution for glucose tolerance (12-14) (Figure 1).

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 outbred controls). In female GK rats, somewhat lower plasma glucose concentrations are noted. Non-fasting plasma insulin levels in GK rats

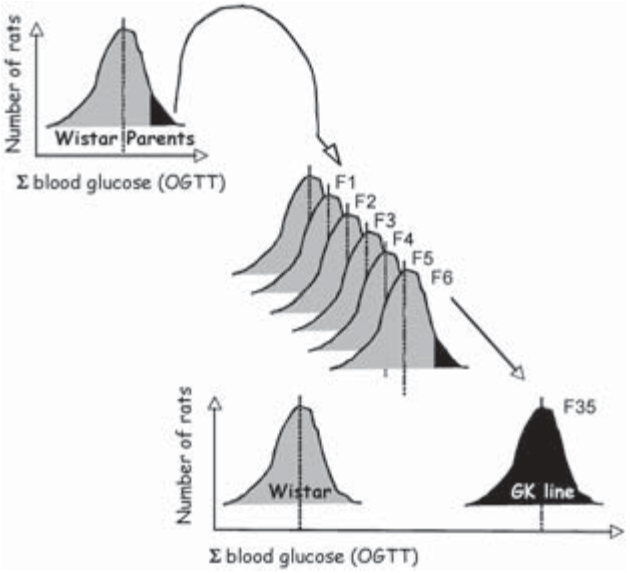

FIGURE 1. ***Design for the production of spontaneously diabetic GK rat by repetition of selective phenotyping****: here is shown the distribution of the sum of blood glucose values (Σ blood glucose) during a standardised oral glucose tolerance test (OGTT) in original parent Wistar rats and F1 to F35 rats. The GK rat line (Wistar strain) has been produced by Goto et al. in Tohoku University, Sendaï, Japan, by selective breeding over many generations from a non diabetic Wistar rat colony on the basis of glucose intolerance (12). The diabetic state was reported to become stable after the 30 generations of selective crosses.*

from all colonies have been found similar or somewhat increased as compared to age-matched controls. During the long-term inbreeding of GK rats (> 20 years) the animals have maintained rather stable levels of glucose intolerance and impairment of glucose-induced insulin response, also when studied in the various sublines of GK rats. However, other characteristics such as beta cell number, insulin content and islet metabolism have been reported to differ between different subline colonies, suggesting that different local breeding environment and/or newly introduced genetic changes account for contrasting phenotypic properties, but it is not clear wether the reported differences are artefactual or true. Signs of early neuropathy (2 mo) have been reported in GK adult while nephropathy and retinopathy develop late (12 mo). The VMH-lesioned GK rat displays accentuated hyperglycaemia and hypertriglyceridaemia with visceral

fat accumulation and both microangiopathy and macroangiopathy. Figure 2 summarizes the GK/Par rat pathogenic sequence culminating in the chronic hyperglycaemia at adult age.

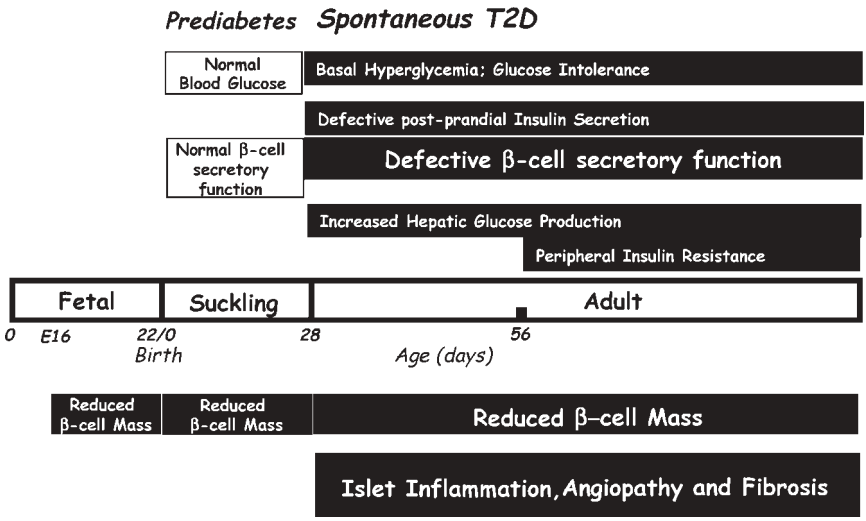

FIGURE 2. ***Time-course of diabetes in the GK/Par rat model.*** *Males and females are similarly affected and their diabetic state is stable over 72 weeks of follow up (13). In adult GK rats, plasma insulin release in vivo in response to i.v. glucose is abolished (13). In vitro studies of insulin release with the isolated perfused pancreas or with perifused islets indicate that both the early and late phases of glucose-induced insulin release are markedly affected in the adult GK rat. Concerning insulin action in adult GK rats, decreased insulin sensitivity has been reported in the liver, in parallel to moderate insulin resistance in extrahepatic tissues (muscles and adipose tissues) (13). Hyperglycaemia is preceded by a period of normoglycaemia, ranging from birth to weaning (15). Therefore during this period the young GK rats can be considered to be prediabetic.*

WHAT IS WRONG IN THE BETA-CELL POPULATION OF THE GK/PAR RAT ONCE DIABETES IS THERE?

Decreased beta-cell number

In the adult GK/Par, total pancreatic beta-cell mass and pancreatic insulin stores are similarly decreased (by 60%) (15). 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 (13). Moreover, the adult GK/Par pancreas exhibits two different populations of islets: large islets which are disrupted by connective tissue (15) and display heterogeneity in the staining of the beta-cells, and small islets with heavily stained beta-cells and normal architecture.

The islets of adult GK/Par rats show decreased beta-cell number and low insulin content compared with control islets. The islet DNA content was decreased to a similar extent, consistent with our morphometric data, which indicates 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 Wistar/Par) islets, which supports some degranulation in the beta-cells of diabetic animals (16).

Moreover, using a complementary approach that associated gene expression analysis (Affymetrix microarrays), quantitative RT-PCR and immunohistochemical studies of pancreata as a function of hyperglycaemia duration, we have recently demonstrated that an inflammatory reaction is associated with islet fibrosis in 4-month-old diabetic GK rats according to a process reminiscent of microangiopathy (17). These alterations worsened with hyperglycaemia duration and might contribute to enhanced GK beta-cell impairment.

Decreased beta-cell population due to early limitation of beta-cell neogenesis

Extensive follow-up of the animals after delivery revealed that GK/Par pups become overtly hyperglycaemic for the first time after three to four weeks of age only. Nevertheless, total beta-cell mass in GK neonates is clearly decreased (by 60%) when compared with Wistar rats (Figure 2) (15). We first previously demonstrated that the early beta-cell growth retardation in the GK/Par model cannot be ascribed to decreased beta-cell replication, nor to increased apoptosis (15). We therefore postulated that the recruitment of new beta-cells

from the precursor pool was defective in the young GK rat. A meaningful set of data from our group (18-20) suggests that the permanently reduced beta-cell mass in the GK rat indeed reflects a limitation of beta-cell neogenesis during early fetal life.

First, the comparative study of the development of GK and Wistar pancreases indicates that the beta-cell deficit (reduced by more than 50%) starts as early as fetal age 16 days (E16) (19). The decreased proliferation and increased apoptosis 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, recent data from our group indicate that defective signalling 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 (21). Low levels of pancreatic of Igf2 associated with beta-cell mass deficiency is maintained thereafter within the fetal pancreas (22) (Figure 3). We also have unpublished data related to crossbreeding protocols between non-diabeticW and diabetic GK rats: at E18.5, Igf2 protein expression is low in GK/GK, W/GK and GK/W pancreata, similar low values in E18.5 crossed W/GK and GK/W fetuses, those values being close to that observed in GK/GK fetuses of the same age. These findings rather support the hypothesis that the pancreatic Igf2 anomaly in the GK diabetic model is linked to a genetic determinism. This view is also consistent with the results of genetic analyses that linked a locus containing the gene encoding *Igf2* to diabetes in the GK rat (23). The *Igf2* gene is subjected to paternal genomic imprinting (24). However, because the *Igf2* expression is similarly affected in fetuses, regardless of whether the father is W or GK, we cannot conclude to a simple change of *Igf2* gene imprinting in the GK rat.

Second, to evaluate the capacity for beta-cell compensatory growth during the neonatal period, we took advantage of the report that the destruction of the beta-cell mass subsequent to streptozotocin injection in the neonatal rat is followed by spontaneous regeneration through both differentiation of precursor cells (neogenesis) and increased proliferation of surviving beta-cells (18). Using such

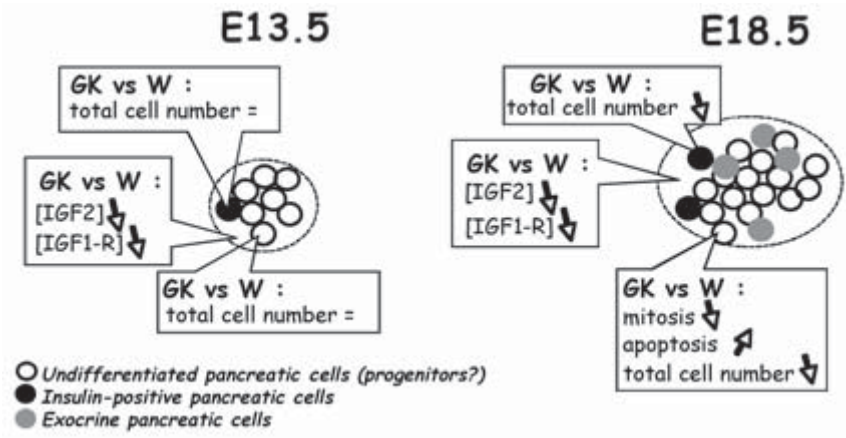

FIGURE 3. ***Schematic presentation of the defective early pancreatic development in the GK/Par rat.*** *Pancreatic cell proliferation and apoptosis and were estimated at embryonic ages E13.5 and E18.5. Comparison of the dorsal pancreatic buds in GK and Wistar (W) rats indicates that the differentiation of the early endocrine cells which appear between E12-E14 is preserved in GK, the number of insulin- or glucagon-expressing cells being similar to Wistar pancreases. Analysis of cell proliferation and apoptosis revealed no differences between Wistar and GK pancreases at this stage. By contrast at E18.5 onward, the number of beta-cells differentiating from the ductal network is reduced by 74% in the GK rat. The apoptotic cells in the E18.5 GK pancreas are not endocrine cells since we did not detect TUNEL-positive cells expressing insulin or glucagon. Moreover these undifferentiated pancreatic cells exhibit a decreased replicative activity. The morphological analysis of the sections suggested that the apoptotic cells in the GK pancreas are ductal cells. IGF-2 protein expression was decreased by 27% and 58% on E13.5 and E18.5 respectively, in GK pancreatic rudiments as compared to W controls. IGF-1R protein expression was decreased by 42% and 34% on E13.5 and E18.5 respectively in GK pancreatic rudiment as compared to W controls. Therefore, the defective IGF2 and IGF1-R protein expression within the GK pancreatic rudiment (E 13.5) precedes the beta-cell mass anomaly. ↑ represents an increase, ↓ represents a decrease, and = means no change.*

approach, we have shown that the regeneration of the beta-cell mass was impaired in streptozotocin-treated newborn GK rats. Because the index of beta-cell proliferation in the streptozotocin-treated GK and Wistar neonates was identical, it was concluded that the impaired beta-cell regeneration of the GK pancreas results from defective neogenesis (18).

Third, similar conclusions were drawn from duct-cell remodelling and beta-cell regeneration investigations after partial pancreatectomy in the adult GK rat (20).

Taken together, these data are consistent with the notion that a poor proliferation and/or survival of the endocrine precursors during foetal, neonatal and adult life will result in poor development of the beta-cell mass, as well as a decreased pool of endocrine precursors in the pancreas, and hence to an impaired capacity of regeneration by neogenesis (either primary in the fetus or compensatory in the newborn and the adult). Finally, the earliest alteration detected in the GK/Par rat targets the size of the beta-cell population. It is conceivable that some genes among those involved in the GK/Par diabetes belong to the subset of genes controlling early beta-cell development.

Functionally defective beta-cells

In adult GK/Par rats, the plasma insulin release *in vivo* in response to intravenous glucose was lacking (12-14). *In vitro* studies of insulin release with the isolated perfused pancreas or with perifused islets indicated that both the early and late phases of glucose-induced insulin release were markedly affected in the adult GK/Par rat. We have shown that impaired glucose-induced insulin release in GK/Par islets was associated with perturbation of multiple intracellular sites. Since this aspect has been recently and extensively reviewed by us and others (13, 14), it will not be further considered in this paper. Figure 4 illustrates a compendium of the abnormal intracellular sites so far identified in the diabetic GK beta-cell.

WHAT ARE THE MECHANISMS THAT UNDERLIE THE PROGRAMMING OF THE GK BETA-CELL DYSFUNCTIONS?

Is beta-cell functional failure due to the abnormal metabolic environment (gluco-lipotoxicity)?

In the GK/Par rat, basal hyperglycaemia and normal to very mild hypertriglyceridaemia are observed only after weaning (13, 15). The onset of a profound alteration in glucose-stimulated insulin secretion by the GK beta-cell (after weaning) is time-correlated with the

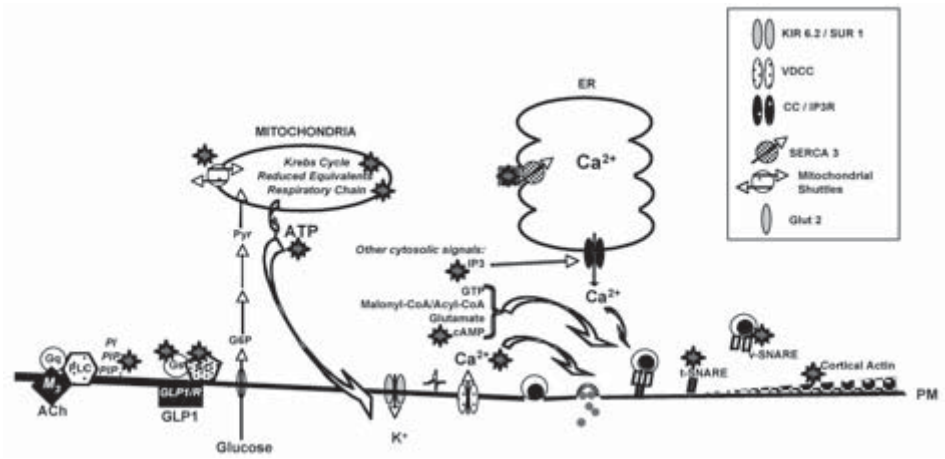

FIGURE 4. ***Model for defective glucose-induced insulin release and the abnormal intracellular sites identified in the diabetic GK beta-cell (13).*** *Impaired insulin response to glucose may be attributed to impaired elevation of intracellular [Ca^{2+}]i, which is a consequence of the failure by glucose to augment L-type Ca^{2+} channel activity, owing 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 Ca^{2+} handling by the endoplasmic reticulum in response to high glucose may also contribute to the defective Ca^{2+} signalling. The sequestration of calcium by endoplasmic reticulum during high glucose exposure (attributed to activation of the calcium-ATPases, SERCAs) may be impaired in the GK beta-cell. Impaired calcium sequestration could also occur because of insufficient cytosolic ATP generation in response to high glucose. In GK islets, glucose fails to increase inositol-phosphate (IP3) accumulation. This is linked to an anomaly in targeting the phosphorylation of phosphoinositides: the activity of phosphatidyl-inositol kinase, the first of the two phosphorylating activities responsible for generating phosphatidyl-inositol biphosphate, is reduced. Moreover, deficient calcium handling and ATP supply in response to glucose probably contributes to abnormal activation of PI kinases and phospholipase C. Concerning cAMP, it is remarkable that its intracellular content is high in GK β-cells already at low glucose. This is related to increased expression of the cyclase isoforms 1, 2, 3 and of the Gsα and Gαolf proteins. Furthermore, cAMP is not further enhanced at increasing glucose concentrations (at variance with the situation in normal beta-cells). Moreover, reduced levels of several exocytotic SNARE proteins including VAMP-2, syntaxin 1A and SNAP-25, have been demonstrated in GK islets. We also recently found that the assembly/disassembly of the cortical actin cytoskeleton beneath the plasma membrane, is functionally abnormal in GK islets. Where data are available, the impaired sites in the GK beta-cell are indicated with a symbol: ✸*
Abbreviations: AC, adenylates cyclases; Gs, Gq: heterotrimeric G proteins; PI kinase, phosphatidylinositol-kinase; SERCA3, type3 calcium-ATPase; SNAP, soluble NSF attachment protein; tSNARE, v-SNARE: SNARE proteins (syntaxin, SNAP-25); VDCC, L-type calcium chanel.

exposure to the diabetic milieu. These changes in islet function can be ascribed, at least in part, to a loss of differentiation of beta-cells chronically exposed to even mild chronic hyperglycaemia and elevated plasma non-esterified fatty acids, a process referred to as «gluco-lipotoxicity». When studied under in vitro static incubation conditions, islets isolated from normoglycaemic (prediabetic) GK/Par pups, amplified their secretory response to high glucose, leucine or leucine plus glutamine to the same extent as Wistar islets (13). Therefore, there does not exist a major intrinsic secretory defect in the prediabetic GK/Par beta-cell, which can be considered as normally glucose-competent at this stage. Such a view is supported by the recent reports that chronic treatment of GK rats with phlorizin partially improved glucose-induced insulin release (25, 26, Bailbé and Portha, unpublished data), while hyperlipidaemia induced by high-fat feeding markedly impaired insulin secretion (27).

Consequently, the lack of beta-cell reactivity to glucose, as seen during the adult period when the GK/Par rats are hyperglycaemic in the basal state, represents an acquired defect mainly related to glucotoxicity.

Which determinants (morbid genes vs environment) for early deficiency of the beta-cell mass in the GK/Par model?

Concerning the potential maternal influence on the development of T2D in the GK model, Gauguier et al. (28) reported that adult offspring of GK females crossed with Wistar males have a more marked hyperglycaemia than adult offspring of Wistar females crossed with GK males, suggesting higher maternal inheritance. However, this conclusion was not confirmed in other studies 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. (29) developed an embryo transfer system to examine more convincingly that major point. First, these authors showed that offspring from GK embryos transferred in the uterus of euglycaemic W mother still develop T2D when adults, therefore highlighting a role for genetic factors. Second, they showed that offspring from W embryos reared in hyperglycaemic GK mothers were significantly hyperglycaemic at adulthood (29).

Thus, in W rats with no genetic risk of diabetes, exposure to hyperglycaemia *in utero* (as seen in the GK pregnant mother) increases the risk of hyperglycaemia in adult life (29), this clearly illustrating a diabetogenic role for the GK intrauterine environment.

Concerning the impact of maternal hyperglycaemia (as induced experimentally in the rat by streptozotocin (30) or glucose infusion (31) on beta-cell function specifically, it has been long recognized that it persists into adult life and the second and third generation offsprings. Another recent illustration is offered by observations in rat pups reared artificially on a high carbohydrate (HC) milk formula (32): such alteration of nutrition during the suckling period only, induces persistent adaptation of energy metabolism in adulthood (obesity, glucose intolerance, impaired insulin secretion) and the HC fed females spontaneously transmitted their metabolic characteristics to their progeny without the pups themselves having to undergo any nutritional treatment (32). Taken as a whole, information from the HC rat pup model (32) and from the offspring of mildly hyperglycaemic rat mothers (33) suggests that hyperglycaemia experienced during the foetal and/or early postnatal life contributes to programming of the endocrine pancreas. Such a scenario also potentially applies to the GK/Par model, as GK/Par mothers are slightly hyperglycaemic through their gestation and during the suckling period. Therefore, the gestational diabetic pattern of the GK/Par mothers may contribute per se (besides inherited disease genes) to establish and/or maintain the transmission of endocrine pancreas programming from one GK/Par generation to the next one. By such mechanism, the GK/Par rat can be viewed as a model of developmental programming for T2D (associated to programming for low beta-cell mass), with a stable transgenerational transmission.

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 rats in the Stockholm colony and are associated to GK beta-cell AC3 over expression and increased cAMP generation (34). The contribution if any, of such a mutation to the GK beta-cell growth defect is so far unknown.

Gauguier et al. (23) 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. (35) using GK from the Stockholm colony. This established the polygenic inheritance of diabetes-related parameters in the GK rat. Both studies found the strongest evidence of linkage between glucose tolerance and markers spanning a region on rat chromosome 1, called Niddm1 locus. While it must be recognized that many of the glucose-controlling locus variants reported (23, 35, 36) were associated in fact with hyperinsulinaemia or enhanced insulin secretion, 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 (37). However, no QTL association with beta-cell mass or beta-cell size is found in the GK/Par rat (Ktorza and Gauguier, unpublished data). Therefore, the likehood 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 the following: does epigenetic perturbation of gene expression occur in the developing GK pancreas and does it contribute to the alteration of early beta-cell growth? igf2 and igf1r genes are good candidates for such a perspective.

WHICH THERAPEUTIC FOR A DECREASED BETA-CELL FUNCTIONAL MASS AS SEEN IN THE GK RAT? A ROLE FOR GLP-1 RECEPTOR AGONISTS

Current therapy of T2D includes a modification of life style such as diet and exercise and the use of a variety of pharmacological agents that target increased insulin secretion, decreased hepatic glucose production and increased insulin action. Despite these approaches, a number of T2D patients may require exogenous insulin. Facilitation of T2D treatment may be obtained through beta-cell transplantation, or on more prospective basis, beta-cell mass expansion after stimulation of beta-cell regeneration/neogenesis in the diabetic patients. Indeed, the emerging understanding of beta-cell growth in the adult, from precursor cells found in the pancreatic ducts, hold the promise of developing new strategies for stimulating beta-cell regeneration. Such approach may involve the delivery of

appropriate growth factors to these progenitor cells to obtain a full beta-cell phenotype. GLP-1 could be one of the most promising candidate for doing so.

GLP-1 is an incretin and is produced by the L-cells of the intestine (review in 38). Since its discovery, GLP-1 has received much attention as a possible new treatment for type 2 diabetes. GLP-1 stimulates insulin secretion and biosynthesis and inhibits glucagon release and both these effects are glucose dependent and therefore represent a very safe way of lowering increased blood glucose (38). A key factor limiting the therapeutic potential of GLP-1 is, however, its very short half-life *in vivo* (38). Therefore, GLP-1 analogs with longer duration of *in vivo* actions have been studied. Exendin-4 (Ex-4; now marketed as an antidiabetic under the name exenatide), a peptide isolated from the salivary secretions of *Heloderma suspectum* is one of them (38). Ex-4 shows 53% amino acid identity to GLP-1 and similar insulinotropic action compared to GLP-1 (38). Lately, it was demonstrated that both GLP-1 and Ex-4 were able to stimulate growth and proliferation of pancreatic beta-cells *in vitro* and *in vivo* in adult rodents (38).

Taking advantage of the opportunity that the GK model is a unique one to test the effect of any pharmacological agent suspected to target the beta cell growth and function under conditions of spontaneous T2D, we evaluated if a GLP-1 or Ex-4 treatment applied during few days in pre-diabetic stage in GK rats would halt or prevent the pathological progression.

Accordingly, we have raised the question of what is the impact of GLP-1 or Ex-4 treatment, first in term of beta-cell mass enlargement and long-term improvement of glucose homeostasis in the GK model. To address this issue, we have investigated the capacity of GLP-1 or Ex-4 treatment to promote beta-cell proliferation in GK rats during the pre-diabetic stage and thereby to prevent the pathological progression of the T2D when animals become adults. To this end, GK rats were submitted to GLP-1 or Ex-4 injection from postnatal day 2 to day 6 only, and their body weight and plasma glucose and insulin levels were examined longitudinally from weaning to adulthood (39). Their beta-cell mass and pancreatic functions were tested on day 7 and later on, at 2 months. Both treatments enhanced,

on day 7, pancreatic insulin contents and total beta-cell mass by stimulating beta-cell neogenesis and beta-cell regeneration. Follow up of biological characteristics from day 7 to adult age (2 months) showed that both treatment exerted long-term favorable influence on beta-cell mass and glycaemic control at adult age (Figure 5). As compared to untreated GK rats, 2-month-old GLP-1 or Ex-4 treated GK rats exhibited significantly decreased basal plasma glucose. Their glucose-stimulated insulin secretion, *in vivo* after intravenous glucose load or *in vitro* using isolated perfused pancreas, were improved. Moreover, plasma glucose disappearance rate was increased in both treated GK groups compared to untreated GK group (39). These findings in the GK model indicate that a GLP-1 or Ex-4 treatment limited to the pre-diabetic period, delays the installation and limits the severity of T2D.

We have also raised the question of what is the acute impact of GLP-1 in term of beta-cell secretory function in the GK model. To that aim, we have determined the effect of an acute GLP-1 exposure on GK islets. We first found that GLP-1 was able to restore the glucose competence of the GK beta-cell with a clear return of the biphasic pattern of insulin release (Figure 6). Then, we have demonstrated that this is by a mechanism which mainly reflects an enhanced efficacy of Ca^{2+} on exocytosis (in the absence of Ca^{2+} elevation) and directly relates to an enhanced production of cAMP and overexpression of AC1, 2, and 3 isoforms and Gsα in the GK beta-cells. Such a sensitization to GLP-1 allows acute normalization of the defective glucose responsiveness of the GK beta-cell (40).

According to our preclinical studies in the GK model, the GLP-1 receptor agonists therefore represent a class of compound with unique property due to their beta-cell replenishing effect in spontaneously diabetic rodents. They may prove invaluable agent not only for treatment of yet installed human T2D but also for its prevention.

In conclusion, the defective beta-cell mass and function in the GK/Par model reflects the complex interactions of three pathogenic players: i) several independent loci containing genes responsible for some diabetic traits (but not decreased beta-cell mass); (ii) gestational metabolic impairment inducing a programming of

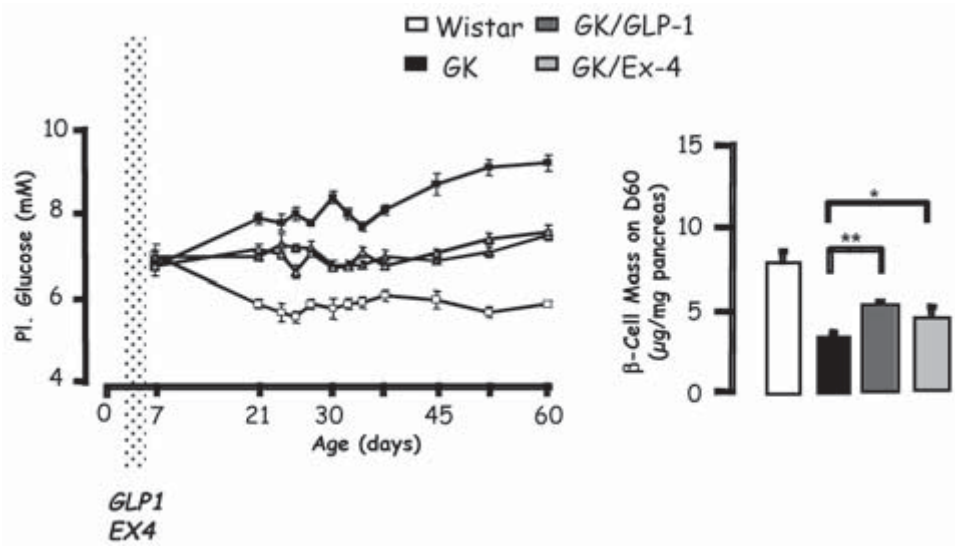

FIGURE 5. ***GLP-1 or Exendin-4 treatment promotes beta-cell proliferation in GK rats during the pre-diabetic stage and thereby prevents the pathological progression of the T2D when animals become adults.*** *Left panel: Evolution of post-absorptive basal plasma glucose levels of W, GK, GK/GLP-1 and GK/Ex-4 from 7 days to 2 months of age. Values are expressed as mean ± SEM. In each group, 10 to 15 animals were studied. Right panel: Total pancreatic beta-cell mass quantification in 60-day-old W, GK, GK/GLP-1 and GK/Ex-4 rats. Values are expressed as mean ± SEM for 4 observations in each group. ** p<0.01; * p<0.05 compared with untreated GK rat.*

endocrine pancreas (decreased beta-cell neogenesis) which is transmitted to the next generation; and (iii) secondary (acquired) loss of beta-cell differentiation due to chronic exposure to hyperglycaemia (glucotoxicity). An important message is that the «heritable» determinants of T2D are not simply dependant of genetic factors, but probably involve transgenerational epigenetic responses.

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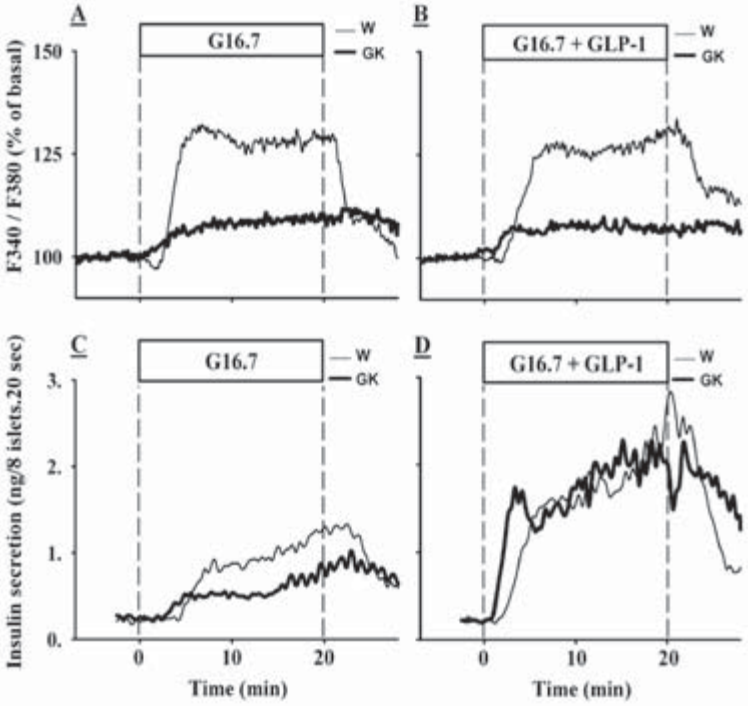

FIGURE 6. ***GLP-1 restores blunted glucose-stimulated insulin secretion by GK islets.*** *Wistar or GK islets were perifused with a medium containing 2.8 mM Glucose (G). G16.7 and GLP-1 were added as indicated. A and B: $[Ca^{2+}]i$ values. C and D: insulin secretion. Data are means of 4 to 8 experiments from three to six different islet preparations in each group.*

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