Fetal Insulin-Like Growth Factor-2 Production Is Impaired in the GK Rat Model of Type 2 Diabetes

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At late fetal age (21.5 days postcoitum [dpc]), GK rats present a severely reduced β-cell mass compared with Wistar rats. This anomaly largely antedates the onset of hyperglycemia in GK rats. Thus, the β-cell mass deficit could represent the primary defect leading to type 2 diabetes in the adult. The aim of this work was to investigate, in GK fetuses at the end of fetal age (21.5 dpc), whether impaired availability of growth factors such as insulin, growth hormone, and IGFs and their IGFBPs could be instrumental in this anomaly. Although it confirms that GK fetuses are hypoinsulinemic despite enhanced plasma glucose level due to maternal hyperglycemia, the present study shows for the first time that IGF-2 expression in the liver and pancreas and IGF-2 serum levels are decreased in GK fetuses. Serum level as well as liver and pancreatic mRNA expression of IGFBP-2 were found to be normal in GK fetuses, whereas serum level and liver mRNA expression of IGFBP-1 were increased. Finally, we found that the maximal β-cell mitogenic response to IGFs in vitro is kept intact, therefore suggesting that the direct biological action of IGFs on fetal GK β-cells is not grossly impaired. In conclusion, in GK fetuses at 21.5 dpc, the defective IGF-2 production appears to be an early landmark in the pathological sequence leading to retardation of β-cell growth in the fetal GK rat.

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RESEARCH DESIGN AND METHODS

Animals and samples. Pregnant GK and Wistar rats were obtained from our local colony. The morning of the discovery of the vaginal plug was taken as day 0.5 dpc. On day 21.5, dpc pregnant rats were anesthetized with pentobarbital sodium (1 ml/kg body wt i.p.; Sanofi Santé Animale, Sanofi, France). Fetal blood samples were collected at the level of the axillary vessels and centrifuged, and plasma (from one fetus) or serum (from two fetuses) were stored at −20°C until assayed. Pancreases (from five fetuses) and a piece of liver (from each fetus) were rapidly excised from fetuses, frozen in liquid nitrogen, and then stored at −70°C until RNA preparation.

Determination of plasma glucose, insulin, and GH levels. Plasma glucose was determined with a glucose analyzer (Beckman Instruments, Fullerton, CA). Immunoreactive insulin plasma was estimated as previously described (8). GH was determined in the plasma of fetuses with a rat GH125I assay system (Biotrak; Amersham Life Science, Amersham, U.K.). The radioimmunoassay was carried out according to the kit protocol. The sensitivity of the assay was 1.6 ng/ml.

Iodination, purification, and determination of serum IGF-1 and -2. Recombinant human IGF-1 and -2 were labeled by a modified chloramine T method (9,10). The specific activity achieved was ~90–175 μCi/μg for both peptides. Before IGF-1 and -2 determination, serum IGFBPs were removed by
Table 1

<table>
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<tr>
<th>Table 1</th>
<th>Biological characteristics of Wistar and GK fetuses at 21.5 dpc</th>
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<tr>
<td>Body weight (g)</td>
<td>Pancreas weight (ng)</td>
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<tr>
<td>Wistar</td>
<td>4.7 ± 0.1 (59)</td>
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<tr>
<td>GK</td>
<td>4.6 ± 0.1 (45)</td>
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Data are means ± SE. Number of determinations is shown in parentheses. Fetuses were obtained from 4–13 different litters. *P < 0.001 and **P < 0.05 as compared with Wistar fetuses.

RESULTS

Biological characteristics of Wistar and GK fetuses at 21.5 dpc

Body weight was similar in the two groups of fetuses (Table 1). GK pancreases used for RNase protection assay (determination of IGF and IGFBP mRNA expression) showed a lower (P < 0.001) weight than Wistar pancreases. By contrast, GK fetuses exhibited a higher plasma glucose concentration and a lower plasma insulin level (P < 0.001) as compared with Wistar fetuses. Plasma GH levels in GK fetuses were slightly increased (P < 0.05) as compared with Wistar levels.

Serum levels of IGFs and IGFBPs in Wistar and GK fetuses

Serum levels of IGF-2 in Wistar fetuses at 21.5 dpc were higher (P < 0.001) than those of IGF-1 in the same rats (128 ± 11 ng/ml, n = 11, vs. 34 ± 4 ng/ml, n = 9) (Fig. 1). Similar serum IGF-1 levels were observed in Wistar and GK fetuses at 21.5 dpc. By contrast, serum IGF-2 levels in GK fetuses were reduced (P < 0.001) by 65% as compared with Wistar levels, and they reached serum values similar to those of IGF-1 (44 ± 6 ng/ml, n = 7, vs. 34 ± 8 ng/ml, n = 8). Serum IGFBP levels were expressed as percent of corresponding Wistar fetuses. Serum IGFBP-1 levels in GK fetuses were increased (P < 0.001) as compared with Wistar fetuses. No change in serum IGFBP-2 was observed between the two groups of fetuses.
Liver IGF and IGFBP mRNA expression in Wistar and GK fetuses. The weight of liver samples used for RNase protection assay (determination of IGF and IGFBP mRNA expression) was similar in Wistar and GK fetuses (121.7 ± 9.9 mg, n = 12, and 105.7 ± 6.7 mg, n = 14, respectively) (Fig. 2).

Densitometric measurements of protected probe fragments are expressed as percent of the corresponding Wistar values. Values are means ± SE for six to seven observations in each group. Fetuses were obtained from three to five different litters. *P < 0.001.

FIG. 2. A: RNase protection assay of liver IGF-1 and -2 mRNA transcripts in Wistar (□) and GK (■) fetuses at 21.5 dpc. B: RNase protection assay of liver IGFBP-1 and -2 mRNA transcripts in Wistar (□) and GK (■) fetuses at 21.5 dpc. *P < 0.05; **P < 0.01.

In vitro mitogenic effect of IGFs in isolated fetal islets. The number of β-cells per isolated fetal islet from GK rats was significantly lower as compared with that in Wistar rats (402 ± 34 β-cell/islet, n = 43, vs. 560 ± 44 β-cell/islet, n = 50, P < 0.01) (Fig. 4). BrdU labeling index of β-cells in absence of IGF was similar in Wistar and GK-isolated fetal islets (1.12 ± 0.18%, n = 15, and 1.27 ± 0.14%, n = 14, respectively). Addition of IGF-1 or -2 to the Wistar-isolated fetal islets significantly increased (1.68 ± 0.19%, n = 14, and 1.68 ± 0.17%, n = 14, respectively, P < 0.05) the β-cell replication above the basal values (without IGF). In Wistar islets, no difference was observed between the in vitro mitogenic effect of IGF-1 or -2. Similarly, addition of IGF-1 or -2 to the GK-isolated fetal islets significantly increased (2.05 ± 0.22%, n = 19, and 2.37 ± 0.25%, n = 17, P < 0.01) the β-cell replication above the basal values (without IGF). Also, no difference was ob-

FIG. 3. A: RNase protection assay of pancreas IGF-1 and -2 mRNA transcripts in Wistar (□) and GK (■) fetuses at 21.5 dpc. Densitometric measurements of protected probe fragments are expressed as percent of the corresponding Wistar values. B: RNase protection assay of pancreas IGFBP-1 and -2 mRNA transcripts in Wistar (□) and GK (■) fetuses at 21.5 dpc. Densitometric measurements of protected probe fragments are expressed as percent of the corresponding Wistar values. 18S ribosomal anti-sense assayed in the same samples are shown beneath the IGF and IGFBP bands; + and − designate riboprobe lanes treated with or without RNases, respectively. Representative experiments are shown in the figure. Values are means ± SE for six to seven observations in each group. Fetuses were obtained from seven to nine different litters. *P < 0.001.
obtained in the absence of IGF.

DISCUSSION

At late fetal age (21.5 dpc), GK rats present a severely reduced β-cell mass compared with Wistar rats (1,2). The aim of this work was to investigate whether impaired availability of growth factors such as insulin, GH, and IGFs and their IGFBPs could be instrumental in this anomaly.

Although it confirms that GK fetuses are hypoinsulinemic despite enhanced plasma glucose level due to maternal hyperglycemia, the present study shows for the first time that IGF-2 expression in the liver and pancreas and IGF-2 serum levels are decreased in GK fetuses.

IGFs are locally produced by pancreas, where they act in an autocrine or paracrine manner and are involved in the regulation of islet growth and differentiation (5). We have previously shown that the reduced β-cell mass in GK fetuses is due to an early impaired rate of β-cell neogenesis, because poor proliferation and/or survival of endocrine precursor cells leads to early defective development of the β-cell mass (3). Thus, the reduced IGF-2 serum level together with the reduced expression of IGF-2 in pancreas of GK fetuses could play a crucial role in the anomaly of β-cell mass in the GK fetus. This is substantiated by the recent demonstration that an increased expression of IGF-2 under the control of the rat insulin promoter in β-cells of transgenic mice led to β-cell hyperplasia (14). In a rat model of increased islet number induced by high-carbohydrate feeding in the neonate, IGF-2 expression was found increased within the pancreatic ductal epithelium, and it may contribute to the associated higher rate of neogenesis (15). There is also clear evidence that IGF-2 inhibits cell apoptosis in many cell types. Hill et al. (16) have demonstrated that increased and persistent circulating IGF-2 in neonatal transgenic mice suppresses developmental apoptosis in the pancreatic islets. Conversely, Petrik and al. (17) have suggested that a reduced pancreatic expression of IGF-2 may contribute to the increased apoptosis seen in the fetus after low-protein diet. However, it is unlikely that the reduced pancreatic expression of IGF-2 observed in GK fetuses may contribute to an increased β-cell death because β-cell apoptosis rate was not increased in GK fetuses at late fetal age (3). Due to our observation that the pancreatic expression of IGF-2 is decreased by 55% in GK fetuses, the anomaly of the IGF-2 expression in pancreas of GK fetuses could be the reflection of the reduced β-cell mass (by 60%) observed at this stage. However, in the present study, we observed that the IGF-1 pancreatic expression is similar in GK and Wistar fetuses, and it is known that IGF-1 is produced by fetal and neonatal rat pancreatic islets (18,19). Therefore, the reduced pancreatic IGF-2 expression in GK fetuses cannot be solely attributed to the decreased β-cell mass observed at this stage. Moreover, whereas pancreatic expression of IGF-2 in normal fetus, as shown by in situ hybridization technique, is largely associated with pancreatic endocrine cells (20), it has also been reported within the pancreatic ductal epithelium in neonatal rat pancreas (15).

Recent studies in our laboratory have suggested that the growth and endocrine differentiation of GK and Wistar pancreatic rudiments are identical when followed in vitro (3). Thus, as far as the in vivo fetal situation is recapitulated by the in vitro development of the GK rudiments, the anomaly of the GK rat leading to deficient pancreatic endocrine cell differentiation in vivo could mainly result from a deficiency of one (or several) extrapancreatic factor(s) (3). Because it is known that in the developing normal pancreas, IGF-2 is involved in the regulation of both islet growth and differentiation (5), the reduced circulating IGF-2 levels in GK fetuses could therefore play a crucial role in the anomaly of β-cell mass in the GK fetuses. As circulating levels of IGF-2 in the rat fetus derive predominantly from the hepatic production site (5), the decreased serum IGF-2 levels are probably the result of the decreased IGF-2 mRNA hepatic expression. Involvement of circulating insulin and plasma glucose levels in the regulation of liver IGFs production at fetal stages has been repeatedly reported (21), whereas the role of plasma GH on fetal IGF regulation is considered as negligible (10,21). Therefore, it is important to take into account the IGF data available in a rat model of induced (streptozotocin) gestational diabetes with fetal hyperglycemia and hypoinsulinemia in the range of the values found in GK fetuses (10). In this study (10), the fetal serum IGF-2 (and IGF-1) level as well as the fetal liver IGF-2 (and IGF-1) mRNA expression were found to be clearly increased, which is in accordance with reports showing that high glucose in vivo (22) or in vitro (23) increases liver IGF-2 expression. The only in vivo situation with a reported decrease of the serum IGF-2 was observed between the in vitro mitogenic effect of IGF-1 and -2 in GK islets.

FIG. 4. BrdU labeling index of β-cells in isolated fetal islets from Wistar (W) and GK rats. Isolated fetal islets obtained after 6 days, culture were further cultured for 2 days without (○) or with 100 ng/ml IGF-1 (▲) or 100 ng/ml IGF-2 (■). Values are means ± SE. BrdU labeling index was determined in each condition in 14–19 isolated fetal islets. Fetal islets were obtained from three to four independent islet cultures. *P < 0.05 and **P < 0.01 as compared with the respective values obtained in the absence of IGF.
found in fetuses from undernourished mothers (10). However, in this last situation, both fetal liver IGF-2 (and IGF-1) mRNA levels were still significantly increased as compared with normal fetuses. Taken together, these observations suggest that the situation in the GK fetuses is a very unique one because the decreased IGF-2 level in the serum and its decreased expression in the liver seems to be largely independent of the variations of fetal insulin and glucose levels. Thus, IGF-2 defective production in the GK fetuses may reflect a primary and generalized anomaly. Such a view is also consistent with the results of previous genetic studies reporting that a locus containing the gene encoding the IGF-2 is associated with diabetes phenotype in the GK rat (24). Whereas defective IGF-2 appears to be an early landmark in the pathological sequence leading to retardation of β-cell growth in the fetal GK rat, the pancreatic IGF-2 status in the aging adult GK animal is quite different. Using the swedish GK colony, Höög et al. (25) have reported that an inappropriately processed IGF-2 of high molecular weight accumulates in pancreas from 6-month-old GK rats. More immunoreactive IGF-2 was localized together with insulin to secretory granules in a subset of large and irregular-shaped islets than in either GK rat islets with normal structure or islets from normal rats (26). Because this increase in the high molecular weight form of IGF-2 in the GK pancreas appears to be a late consequence of the early reduction of β-cell number/function in the GK model, it might be triggered by long-term hyperglycemia and might represent an islet self-damaging process disrupting normal arrangement of islet architecture through fibroblast proliferation and collagen deposition.

In view of the reported ability of IGFBPs to modulate IGF bioactivity, we examined serum and tissular expression of IGFBP-1 and -2 in GK fetuses. In general, IGFBP-2 appears to inhibit IGF actions, particularly those of IGF-2 (4). We report here normal serum, liver, and pancreatic mRNA expression of IGFBP-2 in GK fetuses. This conclusion is not at odds with the general agreement that few changes in liver IGFBP-2 mRNA were found in fetuses from experimental diabetic (10) or undernourished mothers (6). By contrast, an increased serum and liver mRNA expression of IGFBP-1 was found in GK fetuses. Insulin appears to play a major role in regulating IGFBP-1 gene transcription in adult animals, i.e., IGFBP-1 transcription is high in diabetic animals and rapidly reduced to normal values after insulin treatment (27,28). The hypoinsulineemic status of GK fetuses could therefore contribute to the high levels of IGFBP-1.

Finally, we have tested the possibility that direct biological action of IGFs on fetal GK β-cell is impaired. Our in vitro results showed that IGF-1 and -2 stimulate the β-cell replication in fetal Wistar islets in accordance with a previous demonstration (29). Similarly, addition of IGF-1 or -2 to the GK isolated islets significantly increased the β-cell replication. These effects were obtained with a submaximal IGF-2 concentration and a maximal IGF-1 concentration based in our circulating-levels evaluation and in vitro data, respectively (19,30). Therefore, we can conclude that IGF responsiveness by fetal GK islets is maintained but cannot eliminate the possibility that the sensitivity of the islet to these factors could be altered.

In conclusion, in GK fetuses at 21.5 dpc, the defective IGF-2 production appears to be an early landmark in the pathological sequence leading to retardation of β-cell growth in the fetal GK rat.


