Glucose and Insulin Regulate Glycosylphosphatidylinositol-Specific Phospholipase D Expression in Islet β Cells

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Insulin resistance is associated with a compensatory islet hyperactivity to sustain adequate insulin biosynthesis and secretion to maintain near euglycemia. Both glucose and insulin are involved in regulating proteins required for insulin synthesis and secretion within the islet and islet hypertrophy. We have determined that glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD) is present within the secretory granules of islet β cells. To determine if GPI-PLD is regulated in islet β cells, we examined the effect of glucose and insulin on GPI-PLD expression in rat islets and murine insulinoma cell lines. Glucose (16.7 mmol/L) increased cellular GPI-PLD activity and mRNA levels 2- to 7-fold in isolated rat islets and βTC3 and βTC6-F7 cells. Insulin (10^{-7} mol/L) also increased GPI-PLD mRNA levels in rat islets and βTC6-F7 cells 2- to 4-fold commensurate with an increase in GPI-PLD biosynthesis. To determine if islet GPI-PLD expression is increased in vivo under conditions of islet hyperactivity, we compared GPI-PLD mRNA levels in islets and liver from *ob/ob* mice and their lean littermates. Islet GPI-PLD mRNA was increased 5-fold while liver mRNA and serum GPI-PLD levels were reduced 30% in *ob/ob* mice compared with lean littermate controls. These results suggest that glucose and insulin regulate GPI-PLD mRNA levels in isolated islets and β-cell lines. These regulators may also account for the increased expression of GPI-PLD mRNA in islets from *ob/ob* mice, a model of insulin resistance and islet hyperactivity.

INSULIN RESISTANCE IS associated with a compensatory islet hyperactivity to sustain adequate insulin biosynthesis and secretion to maintain near euglycemia. Increased expression of additional proteins within the β cells also occur and include the insulin processing proteases PC2 and PC3[1]a nd islet amyloid polypeptide.[2] The increase in β-cell protein expression may be mediated in part by glucose or insulin in an autocrine fashion.[3-5]

We and others have determined that glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD) is present within islet β cells.[6,7] GPI-PLD is most abundant in serum where it is a high-density lipoprotein (HDL)-associated protein.[8,9] GPI-PLD mRNA is expressed in many tissues including β cells, macrophages, keratinocytes, and liver.[10] Liver has the highest level of mRNA levels and likely is the primary source of circulating GPI-PLD.[11-13] Curiously, the amino acid sequence of human serum GPI-PLD corresponds to the human pancreas and not the human liver GPI-PLD cDNA sequence,[14,15] suggesting that islets may contribute to circulating GPI-PLD.

The function of GPI-PLD in serum or cells is not entirely clear. Transient transfection of cells with GPI-PLD is associated with increased release of GPI-anchored proteins from intracellular compartments.[16,17] This generates phosphatidic acid, which is converted to diacylglycerol, leading to activation of protein kinase Cα.[18] Release of cell surface GPI-anchored proteins also appears to occur via a GPI-PLD-mediated cleavage in numerous cell types.[18-20] In islet β cells, GPI-PLD is present in 2 different subcellular compartments, in a membrane fraction (unpublished observation), and within the insulin secretory granule.[7] However, the role of GPI-PLD in β-cell function and whether GPI-PLD expression is regulated in β cells similar to other secretory granular proteins is unknown. To determine if islet expression of GPI-PLD is regulated similar to other secretory granular proteins, we examined the effect of glucose and insulin on GPI-PLD expression in isolated rat islets and βTC6-F7 cells. In addition, we compared GPI-PLD mRNA levels in islets from lean and *ob/ob* mice, a model for insulin resistance and islet hypertrophy.

MATERIALS AND METHODS

Animals

Ob/ob mice and lean littermate controls (male, 8 to 12 weeks of age) were obtained from Dr Dale Romsos, Michigan State University, East Lansing, MI. *Db/db* mice were purchased from Jackson Laboratory, Bar Harbor, MA (male, 8 to 12 weeks of age). Sprague-Dawley rats (male, 200 to 250 g) were obtained from Harlan (Indianapolis, IN). All animal studies were approved by either the Indiana University or Michigan State University Institutional Animal Care and Use Committees. Blood was collected from all mice after a 4-hour fast and serum frozen at –70°C until assayed.

Islet Preparations and Cell Culture

Mouse and rat islets were prepared as previously described.[18] βTC3 and βTC6-F7 cells[21] were a kind gift from Dr Shimon Efrat, Albert Einstein College of Medicine, New York, NY. Cells were cultured as previously described.[7] Total RNA was extracted, and Northern blotting for GPI-PLD mRNA using a full-length cDNA was performed as previously described.[22]

To measure GPI-PLD biosynthesis, βTC6-F7 cells were incubated in Dulbecco's modified Eagle's medium (DMEM) containing 1 mg/mL of bovine serum albumin (BSA) in the absence or presence of 10^{-7} mol/L insulin for 24 hours. Cells were washed with amino acid–free media and proteins labeled with [^{35}S]methionine/cysteine (250 μCi/mL; American Radiolabeled Chemicals, St Louis, MO). After 60 minutes of

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labeling, cells (60-mm dish) were lysed (50 mmol/L HEPES pH 8.0, 1% vol/vol Triton X-100, 10 mmol/L phenylmethylsulfonyl fluoride, 10 mmol/L pepstatin, 10 mmol/L Na-p-tosyl-l-lysine chloromethyl ketone, 0.1 mmol/L leupeptin, 10 mmol/L aprotinin, and 0.1% vol/vol NaN_3) and GPI-PLD immunoprecipitated with 612c (5 μg), a monoclonal antibody to GPI-PLD.[23] 612c was a gift from Dr Michael Davitz, Darby and Darby, New York, NY. Immunoprecipitates were separated by a 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and visualized by autoradiography.

Analyses

GPI-PLD activity was determined using [^{3}H] myristate-labeled variant surface glycoprotein from *Trypanosoma brucei* as previously described.[22] GPI-PLD immunoreactivity in serum and liver mRNA steady state levels were determined as previously described.[24] Apolipoprotein (apo) AI was determined as previously described.[24] Serum lipoproteins were separated by fast protein liquid chromatography (FPLC) using a Superose 6 column (Amersham Pharmacia, Piscataway, NJ) as previously described.[24] Glucose, cholesterol, and total triglycerides (which includes glycerol) were determined using kits from Sigma (St Louis, MO). Insulin and glucagon assays were conducted by the Indiana University Diabetes Research and Training Center Immunoassay Core using RIA kits from Linco (St Charles, MO). Groups were compared using an unpaired 2-tailed Student's t test with $P < .05$ considered statistically significant.

RESULTS AND DISCUSSION

To determine if glucose regulates GPI-PLD expression in islets and β cells, isolated rat islets and βTC3 cells, a murine β-cell line, were incubated with 5.5 or 16.7 mmol/L glucose. After 48 hours, GPI-PLD activity in rat islets and βTC3 cells increased by 2.3- and 7-fold (Fig 1), respectively. The difference in the magnitude of the glucose effect may reflect differences in glucose responsiveness in islets compared with a cell line and/or the presence of α cells in islets. Because some cell types take up serum GPI-PLD,[25] we examined the effect of glucose on mRNA levels. High glucose (16.7 mmol/L) increased GPI-PLD mRNA levels 2- to 3-fold (data not shown), suggesting that at least some of the increase in cellular GPI-PLD activity is related to increased synthesis.

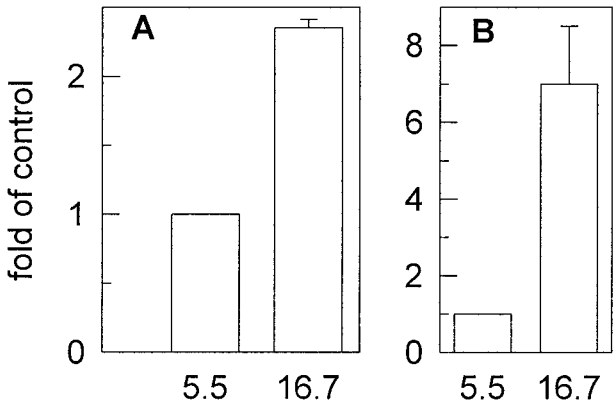

Fig 1. Effect of glucose on cellular GPI-PLD activity in rat islets and βTC3 cells. (A) Rat islets were isolated and incubated for 24 hours in DMEM supplemented with 10% fetal calf serum and the indicated concentration of glucose. (B) βTC3 cells were in 35-mm dishes and incubated in RPMI 1640 supplemented with 10% fetal calf serum containing 5.5 or 16.7 mmol/L glucose. After 48 hours, the media was removed, cells washed with serum-free media, and the cellular GPI-PLD activity determined as described in Materials and Methods. Results represent mean ± SD (n = 3).

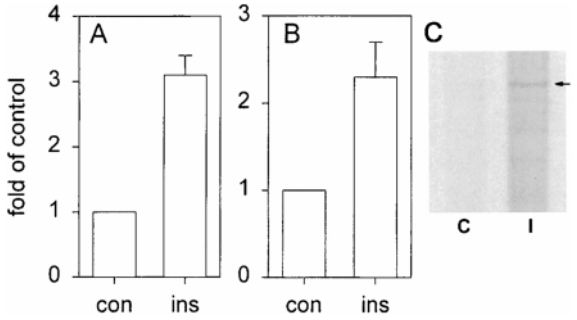

Fig 2. Insulin increases GPI-PLD mRNA steady-state levels and biosynthesis. (A) Rat islets (400 islets) were incubated in DMEM containing 1 mg/mL of BSA in the absence (con) or presence of 10^{-7} mol/L insulin (ins). After 24 hours, GPI-PLD mRNA levels were determined by Northern blotting. Results represent mean ± SD (n = 3). (B) βTC6-F7 cells were treated as in (A) and GPI-PLD mRNA levels determined. Results represent mean ± SD (n = 3). (C) βTC6-F7 cells were treated as in (A) and biosynthesis of GPI-PLD determined as described in Materials and Methods. Location of GPI-PLD indicated by the arrow (115 kd).

Because the effects of glucose on protein expression may be mediated, in part, by an autocrine action of insulin, we examined the effect of insulin on GPI-PLD expression in islets and βTC6-F7 cells. βTC6-F7 cells were chosen because this cell line has a lower rate of constitutive secretion of insulin compared with βTC3 cells (data not shown). Insulin (10^{-7} mol/L) increased GPI-PLD mRNA levels by 3- and 2.3-fold in isolated rat islets and βTC6-F7 cells (Fig 2). This increase in GPI-PLD mRNA levels is associated with an increase in GPI-PLD biosynthesis (Fig 2).

To determine if GPI-PLD expression is increased in vivo under conditions of hyperinsulinemia, we compared GPI-PLD mRNA levels in islets from *ob/ob* and lean littermates. As expected, *ob/ob* mice had higher levels of serum insulin (9-fold) with a trend for higher glucose levels, indicative of increased β-cell mass and insulin resistance in *ob/ob* mice (Table 1). In isolated islets, GPI-PLD mRNA levels were 5-fold higher in *ob/ob* mice compared with lean littermates (Fig 3).

To determine if the increased expression of islet GPI-PLD may affect serum levels, we compared serum GPI-PLD levels in *ob/ob* and lean mice. In *ob/ob* mice, total serum cholesterol (45%), and apo AI (4.5-fold), the major protein in HDL, were higher than in lean littermates (Table 1). FPLC analysis of serum demonstrated that GPI-PLD was associated with HDL in both *ob/ob* and lean mice (data not shown). This is consistent with the human GPI-PLD–containing particle, which also includes apo AI- and AIV.[9] Although apo AI levels were increased, serum GPI-PLD levels were decreased by 33% (Table 1). Similarly, liver GPI-PLD mRNA levels were also lower in *ob/ob* mice by nearly 75%. *Db/db* mice also have 60% lower GPI-PLD mRNA levels compared with lean littermates (data not shown), suggesting that the decrease in liver GPI-PLD

Table 1. Summary of Metabolic and GPI-PLD Levels in Lean and ob/ob Mice

	Lean (5)	ob/ob (5)
Glucose (mg/dL)	131 ± 23	171 ± 57
Insulin (ng/dL)	0.41 ± 0.02	3.6 ± 3.0*
Glucagon (ug/dL)	43 ± 38	33 ± 20
Cholesterol (mg/dL)	85 ± 8	122 ± 16*
Total triglycerides (mg/dL)	109 ± 34	93 ± 20
Apo AI (mg/mL)	1.6 ± 0.2	7.5 ± 1.2*
GPI-PLD		
Serum activity (U/μL)	20 ± 4	16 ± 5
Serum mass (fold of control)	1.0 ± 0.2	0.66 ± 0.29*
Liver mRNA (fold of control)	1.0 ± 0.49	0.27 ± 0.16*

NOTE. Values represent mean ± SD.
* $P < .05$.

mRNA levels are not due to a deficiency in leptin or leptin signaling. The parallel changes between serum levels and liver mRNA levels are consistent with liver as a major source of circulating GPI-PLD.

These results suggest that GPI-PLD expression within islets is regulated by glucose, similar to other secretory granule proteins, and that this may occur via an autocrine effect of insulin. Insulin regulation of GPI-PLD mRNA levels may explain our observation that both serum and a serum substitute, Nutridoma (Boehringer Mannheim, Indianapolis, IN), which contains high concentrations of insulin, increase GPI-PLD mRNA levels in βTC6-f7 cells (data not shown). The role of GPI-PLD in β-cell function is unclear at this point. GPI-PLD may have different functions depending upon its subcellular localization. In β cells, GPI-PLD is both membrane bound (unpublished observation) and in the insulin secretory granule.[7] Membrane bound GPI-PLD may cleave GPIs intracellularly.[18] In the endoplasmic reticulum, GPI-PLD–mediated cleavage of GPIs increases diacylglycerol levels (derived from the phosphatidic acid generated) and activates protein kinase Cα.[18] Hence, membrane bound GPI-PLD may have a role in stimulus-secretion coupling. Conversely, GPI-PLD within the secretory granule may have a different function, as GPI-PLD affects islet amyloid polypeptide fibril formation in vitro (Deeg et al, manuscript in preparation).

Although GPI-PLD is secreted from islets, it does not appear to make a significant contribution to the circulating levels of GPI-PLD. In fact, patients with total pancreatectomy have normal levels of serum GPI-PLD activity (data not shown). The regulation of serum GPI-PLD appears complex as changes in serum GPI-PLD levels appear independent of changes in the bulk of HDL. We observed that high-fat diets that decreased HDL levels in C57BL/6 mice have no effect on serum GPI-PLD levels.[22] Conversely, we observed here that despite 5-fold increases in apo AI, there was a concomitant decrease in serum GPI-PLD levels in *ob/ob* mice. This is consistent with the observation that GPI-PLD is associated with a small, minor discrete HDL, which in humans, accounts for less than 0.2% of the total HDL.[9] This also suggests that the metabolism of this small, GPI-PLD-containing HDL differs from the bulk of HDL. In our studies with mouse models of diabetes, the changes observed in serum paralleled the changes in liver mRNA with the opposite changes occurring in the islet, ie, increased serum mass and liver mRNA with decreased islet GPI-PLD with islet destruction and decreased serum mass and liver mRNA with increased islet GPI-PLD with hyperinsulinemia. Thus, the rate of GPI-PLD synthesis in the liver may influence the serum levels of HDL. It is unclear at this point what regulates liver expression of GPI-PLD, but the discordance between liver and islet expression of GPI-PLD in the mouse models of diabetes suggests tissue specific regulation. GPI-PLD has been reported to be increased in skeletal muscle in *ob/ob* mice.[26] However, it is unlikely that the mRNA identified by subtraction cloning corresponds to a protein with GPI-PLD activity, because the mRNA transcript was 1.1 kb, which is much smaller than any reported GPI-PLD mRNA species and encoded a protein that corresponds to the β propeller and not the catalytic domain of known GPI-PLDs (D. Vicent, personal communication, September 1998).

In summary, glucose and insulin increase GPI-PLD mRNA levels in isolated islets and β-cell lines. These regulators may account for the increased expression of GPI-PLD mRNA in islets from *ob/ob* mice, a model of insulin resistance and islet hyperactivity. Further studies are underway to examine the role of GPI-PLD in β-cell function.

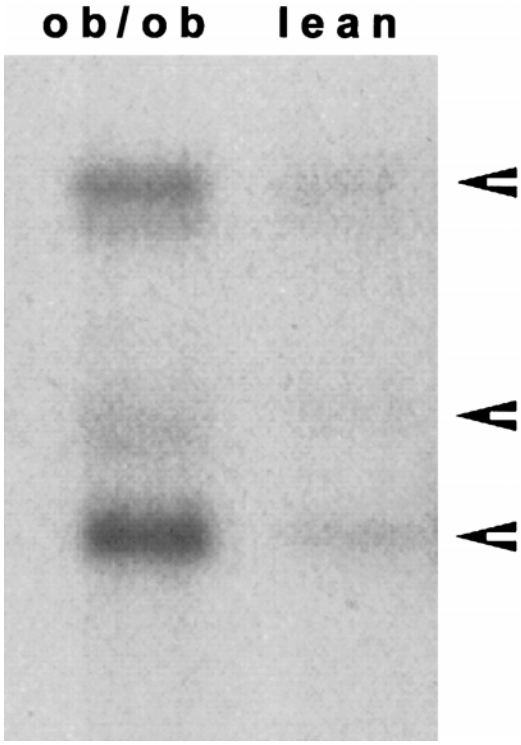

Fig 3. GPI-PLD mRNA levels in islets from ob/ob and lean mice. Islets were harvested from *ob/ob* and lean mice, 5 animals from each group, and GPI-PLD mRNA identified by Northern blotting as described in Materials and Methods. Ethidium bromide staining of 28S and 18S was similar in *ob/ob* and lean islets. Arrows identify the 3.9, 5.4, and 8.0 transcripts.

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