Defective Stimulus-Secretion Coupling in Islets of *Psammomys obesus*, an Animal Model for Type 2 Diabetes

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***Psammomys obesus* is a model of type 2 diabetes that displays resistance to insulin and deranged β-cell response to glucose. We examined the major signaling pathways for insulin release in *P. obesus* islets. Islets from hyperglycemic animals utilized twice as much glucose as islets from normoglycemic diabetes-prone or diabetes-resistant controls but exhibited similar rates of glucose oxidation. Fractional oxidation of glucose was constant in control islets over a range of concentrations, whereas islets from hyperglycemic *P. obesus* showed a decline at high glucose. The mitochondrial substrates α-ketoisocaproate and monomethyl succinate had no effect on insulin secretion in *P. obesus* islets. Basal insulin release in islets from diabetes-resistant *P. obesus* was unaffected by glucagon-like peptide 1 (GLP-1) or forskolin, whereas that of islets of the diabetic line was augmented by the drugs. GLP-1 and forskolin potentiated the insulin response to maximal (11.1 mmol/l) glucose in islets from all groups. The phorbol ester phorbol myristic acid (PMA) potentiated basal insulin release in islets from prediabetic animals, but not those from hyperglycemic or diabetes-resistant *P. obesus*. At the maximal stimulatory glucose concentration, PMA potentiated insulin response in islets from normoglycemic prediabetic and diabetes-resistant *P. obesus* but had no effect on islets from hyperglycemic *P. obesus*. Maintenance of islets from hyperglycemic *P. obesus* for 18 h in low (3.3 mmol/l) glucose in the presence of diazoxide (375 μmol/l) dramatically improved the insulin response to glucose and restored the responsiveness to PMA. Immunohistochemical analysis indicated that hyperglycemia was associated with reduced expression of α-protein kinase C (PKC) and diminished translocation of λ-PKC. In summary, we found that *1*) *P. obesus* islets have low oxidative capacity, probably resulting in limited ability to generate ATP to initiate and drive the insulin secretion; *2*) insulin response potentiated by cyclic AMP–dependent protein kinase is intact in *P. obesus* islets, and increased sensitivity to GLP-1 or forskolin in the diabetic line may be secondary to increased sensitivity to glucose; and *3*) islets of hyperglycemic *P. obesus* display reduced expression of α-PKC and diminished translocation of λ-PKC associated with impaired response to PMA. We conclude that low β-cell oxidative capacity coupled with impaired PKC-dependent signaling may contribute to the animals' poor adaptation to a high-energy diet. *Diabetes* 50:308–314, 2001**

The gerbil *Psammomys obesus* seems an excellent natural model of type 2 diabetes: normoglycemic in its natural habitat, feeding on the low-energy salt bush (*Atriplex halimus*), it shows a high tendency to develop diabetes when fed an energy-rich laboratory diet (1–3). Diet-induced hyperglycemia in *P. obesus* is initially associated with hyperinsulinemia, a significant fraction of which consists of insulin precursor molecules (4,5). β-Cell insulin content is depleted and, with continued hyperglycemia, β-cell mass also is decreased (6). The genetic background for this nutrition-evoked diabetes was demonstrated in the Jerusalem colony of *P. obesus* by selection of two lines of animals, a diabetes-prone (DP) and a diabetes-resistant (DR) line (3). Almost all animals of the DP line develop diabetes when changed from a low-energy (LE) to a high-energy (HE) diet, with 90% developing hyperglycemia within 5 days of HE nutrition (7). Conversely, 60–70% of the animals of the DR line of *P. obesus* are resistant to diet-induced diabetes and remain normoglycemic on an HE diet. Because animals of both lines seem equally resistant to insulin action (8), we studied the relative contributions of genetic predisposition and nutritional intake to β-cell dysfunction and demonstrated a species-dependent defect in insulin release in both lines of *P. obesus* (7). In the DP line, superimposed on this deficiency was a hyperglycemia-induced reduction of the glucose threshold for insulin release with augmented glucose phosphorylation, which promoted depletion of β-cell insulin stores (7). Because very little information is available on β-cell stimulus-secretion coupling in *P. obesus*, the present study was undertaken to examine the major β-cell–signaling pathways of glucose-dependent and -independent insulin release and their modification in relation to diet as well as to diabetic predisposition. A comprehensive evaluation of the dynamics of glucose-dependent calcium metabolism in *P. obesus* islets will be discussed in a separate study. The derangements in coupling pathways reported here should provide the grounds for future studies on the specific molecular events involved.

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BSA, bovine serum albumin; DP, diabetes-prone; DR, diabetes-resistant; GLP-1, glucagon-like peptide 1; HE, high-energy; KIC, ketoisocaproate; KRBB, Krebs-Ringer bicarbonate buffer; LE, low-energy; PKA, cyclic AMP–dependent protein kinase; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; RACK, receptor for activated C kinase; RIA, radioimmunoassay.

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RESEARCH DESIGN AND METHODS

Animals. *P. obesus* (age 2.5–3.5 months) and SD rats (age 1.5–2 months) were obtained from Harlan (Jerusalem, Israel). The DP and DR lines of *P. obesus* were generated by assorted breeding at the Hebrew University Animal Facility (3). To maintain normoglycemia (nonfasted blood glucose <8.3 mmol/l), DP *P. obesus* were fed an LE diet (2.38 kcal/g; Koffolk, Petach Tikva, Israel) from weaning. To induce diabetes, DP *P. obesus* were switched to an HE diet (2.93 kcal/g; Weizmann Institute of Science, Rehovot, Israel) for 5 days. DR *P. obesus* were maintained on an HE diet from weaning; animals with four nonfasted blood glucose levels <8.3 mmol/l were accepted as nondiabetic and included in the study. In studies in which DR animals on an LE diet were used, animals were switched to an LE diet 2 weeks before the experiments.

Islet isolation. Islets were isolated by collagenase digestion (Serva Feinbiochemica, Heidelberg, Germany) and hand picked, as previously described (4).

Insulin response studies. Islet insulin response to secretagogues was evaluated by static incubation in a tissue culture incubator in four-chamber culture plates (Nunclon Delta Multidishes; Nunc A/S, Roskilde, Denmark) with five islets/chamber and 1.0 ml Krebs-Ringer bicarbonate buffer (KRBB), which was extensively gassed with O_2/CO_2 (95%/5%) and supplemented with 0.5% bovine serum albumin (BSA; fraction V). Islets were preincubated for 60 min in batch, then transferred to the individual chambers containing the test reagents in KRBB and 0.5% BSA and incubated for an additional 60 min. Glucose at 2 mmol/l was used as the basal nonstimulatory level, as well as during the 60-min period of preincubation, as higher concentrations were shown to be stimulatory for islets of DP-HE *P. obesus* (7). In some studies (see RESULTS), islets from DP-HE *P. obesus* were cultured overnight in RPMI containing 3.3 mmol/l glucose, 375 µmol/l diazoxide, 2 mmol/l glutamine, 100 U/ml penicillin G, 100 µg/ml streptomycin, and 10% fetal calf serum, then tested as above. Preliminary studies demonstrated that overnight maintenance of islets from DP-HE *P. obesus* in low glucose and diazoxide significantly improved their insulin response to glucose as well as doubled their (nearly depleted) insulin stores (7). At the end of the test period, the plates were placed on ice, the medium was carefully removed and stored at –20°C, and the islets were transferred to a tube containing 1.0 ml of 0.1 mol/l HCl and 0.1% BSA. Following three cycles of freeze-thawing, the fragmented islets were extracted overnight in acid-alcohol at 4°C and centrifuged for 10 min in a microfuge at 14,000 rpm; the supernatant was frozen for analysis of insulin by radioimmunoassay (RIA).

Analysis of glucose utilization and oxidation. Glycolytic flux was determined using rates of production of ^3H_2O from [5-^{3}H]glucose (100 mCi/mmol; DuPont-NEN, Boston, MA). The rate of glucose oxidation was determined by production of $^{14}CO_2$ from [U-^{14}C]glucose (3.4 mCi/mmol; Du Pont-NEN), as previously described (9), with these minor modifications: after a 60-min preincubation, three sets of duplicate batches of 25 islets from each animal were incubated at 37°C for 90 min in KRBB-BSA buffer containing 1.6, 5.5, or 11.1 mmol/l glucose and 2.0 µCi of [5-^{3}H]glucose and [U-^{14}C]glucose. In studies examining the inhibitory effects of fatty acid oxidation (12,13) on rates of glucose utilization and oxidation, the islets were preincubated for 1 h in the presence of 0.4 mmol/l bromopalmitate (2-bromohexadecanoic acid; Aldrich Chemical, Milwaukee, WI) and 0.5% defatted BSA, followed by a 90-min incubation as described above in buffer containing 0.1 mmol/l bromopalmitate in 0.5% defatted BSA; controls were treated with 0.5% defatted BSA alone. Incubations were performed in microfuge tubes placed inside scintillation vials. The reaction was stopped by adding 0.1 ml 10% perchloric acid into the inner tube and a mixture of 0.25 ml water and 0.25 ml hydroxide of hyamine (Packard Instrument, Meriden, CT) in the outer scintillation vial. After a further 24-h incubation, the inner tube was removed and the amount of radioactive water and CO_2 that had diffused into the outer vial determined after addition of 10 ml Ultima Gold scintillation solution (Packard) in a scintillation counter (Packard Tri-Carb 1500) using double-channel setup. Buffer blanks containing 2.0 µCi each of [^{14}C]$NaHCO_3$ (55 mCi/mmol; American Radiolabeled Chemicals) and tritiated water (100 mCi/mmol; Du Pont-NEN) were used to obtain fractional diffusion rates; routine recoveries were 94–96 and 28–35%, respectively.

Immunohistochemical imaging. Pancreatic sections were fixed, treated, and stained as previously described (10). Protein kinase C (PKC) isoenzyme antisera were obtained from Sigma Biochemicals (Rehovot, Israel), Research and Diagnostics Antibodies (Berkeley, CA), and Santa Cruz Biotechnology (Santa Cruz, CA). Cy-3–conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) was used as the secondary antibody. β-Cells were identified by counterstaining with guinea pig anti-insulin (Sigma) sandwiched with Cy-5–conjugated goat anti–guinea pig IgG (Jackson). Imaging was performed using a Laser-Scanning Confocal microscope (MRC-1024; BioRad Microscopy Division, Hemel Hampsted, Herts, U.K.). Three to five pancreata of each DR-HE, DP-LE and DP-HE *P. obesus* were examined, with 3–5 fields being scanned in each section. For each event of isoenzyme determination, slides of all three groups were stained and mounted simultaneously to minimize variability due to staining procedure. Relative levels of PKC isoenzymes were determined using the Image-Pro Plus program (Media Cybernetics, Silver Spring, MD), maintaining identical imaging parameters for each isoenzyme.

Insulin assay. The insulin RIA was performed using anti-insulin coated tubes (ICN Pharmaceuticals, Costa Mesa, CA) and ^{125}I-labeled insulin (Linco Research, St. Charles, MO). Human insulin standard (Novo-Nordisk, Bagsvaerd, Denmark) was used for the *P. obesus* insulin RIA; cross-reactivity and dilution linearity were comparable to those previously determined (11). The routine inter- and intra-assay coefficients of variation were 4–6% and 6–10%, respectively. Hyperglycemic DP *P. obesus* produce varying amounts of insulin, proinsulin, and proinsulin-related products (4), all of which cross-reacted with our assay antiserum; hence as used in this study, the term insulin indicates the sum of these products.

Statistical analyses. Paired nonparametric Wilcoxon or Mann-Whitney rank tests were used to determine level of significance where groups of data were compared. An unpaired Student's *t* test was used to compare means of different experiments. Kruskal-Wallis nonparametric analysis of variance, followed by Dunn's multiple comparisons tests, were applied to analysis of rates of glucose utilization and oxidation. Data were evaluated with the InStat statistical software from GraphPad Software (San Diego, CA).

RESULTS

Metabolic fluxes in *P. obesus* islets. Glycolytic and oxidative fluxes are believed to generate the initial signals for glucose-induced insulin release. Rates of glucose utilization were superimposable in islets from all normoglycemic *P. obesus* (DR-LE, DR-HE, and DP-LE) (Fig. 1*A*), whereas islets from hyperglycemic *P. obesus* (DP-HE) exhibited higher rates, reaching twofold at 11.1 mmol/l glucose, the maximal stimulatory concentration of the sugar (7). On the other hand, no statistical difference was observed in rates of glucose oxidation to CO_2 among any of the groups, although islets from the hyperglycemic animals demonstrated somewhat higher rates at 11.1 mmol/l glucose (Fig. 1*B*). The fractional glucose oxidation (Fig. 1*C*) was concentration-independent in islets from all three groups of normoglycemic *P. obesus;* by contrast, in islets from hyperglycemic DP-HE *P. obesus*, fractional glucose oxidation declined progressively from 22.6 ± 1.5 to 13.0 ± 0.9% between 1.7 and 11.1 mmol/l glucose ($P < 0.0002$) (Fig. 1*C*).

To rule out the possibility that enhanced oxidation of fatty acids may contribute to the decline in fractional glucose oxidation DP-HE *P. obesus* islets, these islets were treated for 60 min with bromopalmitate (0.4 mmol/l) followed by assessment of rates of glucose utilization and oxidation in the presence of 0.1 mmol/l of the inhibitor of fatty acid oxidation (12,13). Islets of the same animal treated with albumin alone served as controls. Paired analysis (five separate experiments) revealed no significant difference in rates of glucose utilization, glucose oxidation, or fractional oxidation of the hexose (fractional oxidation rates were 23.6 ± 4.2 vs. 25.4 ± 3.6%, 16.4 ± 5.5 vs. 23.1 ± 5.9%, and 13.2 ± 2.7 vs. 13.2 ± 2.4% for islets treated with bromopalmitate vs. controls at 1.65, 5.5, and 11.1 mmol/l glucose, respectively).

The possibility of a mitochondrial defect was further tested by examining the insulinotropic efficiency of ketoisocaproate (KIC) and monomethyl succinate, two agents known to be exclusively metabolized by mitochondria in islet cells. Figure 2 shows that both KIC (Fig. 2*A*) and monomethyl succinate (Fig. 2*B*) had minimal or no effect on insulin secretion; higher concentrations (10 or 20 mmol/l) were even less effective (data not shown). In comparison, 5.0 mmol/l KIC and monomethyl succinate augmented insulin release in rat islets by 7.2- and 6.4-fold, respectively.

To further differentiate the effects of hyperglycemia from those of genetic predisposition on insulin response to glucose

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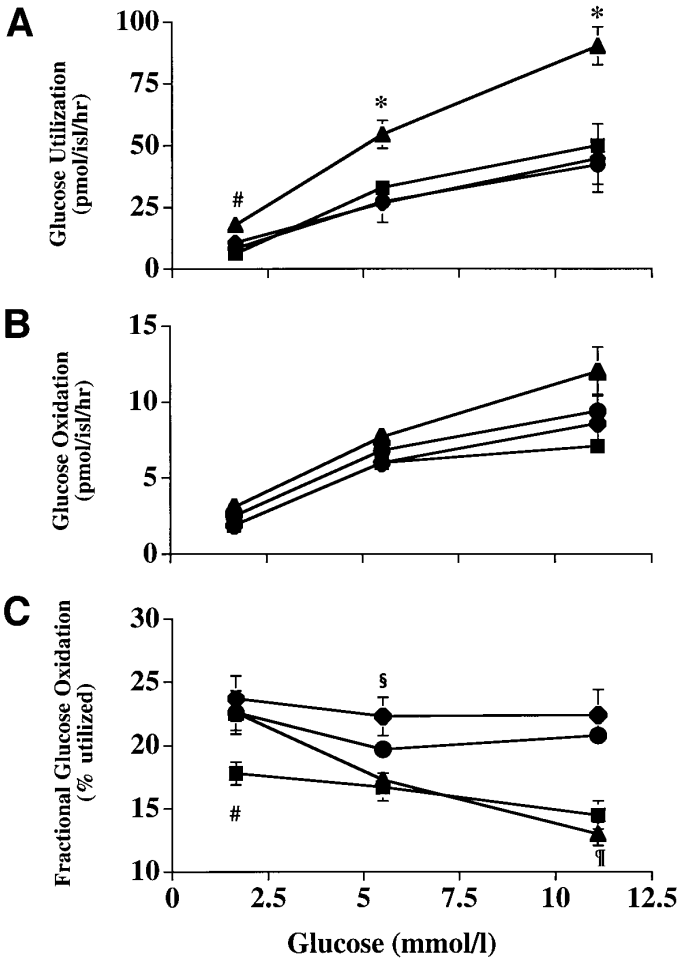

FIG. 1. Glycolytic and oxidative metabolism of glucose in isolated *P. obesus* islets. Rates of glucose utilization (*A*) and oxidation (*B*) were determined in freshly isolated islets from DR-LE (◆) or DR-HE (■) *P. obesus* and from DP *P. obesus* maintained for 5 days on an HE diet (DP-HE; ▲) or continuously fed an LE diet (DP-LE; ●). *C*: Fraction of glucose oxidized to CO_2 by the islets. Each point is the mean of 5–8 experiments ± SE. $P < 0.05$ for *DP-HE vs. DP-LE, DR-HE, or DR-LE; #DP-HE vs. DR-HE; §DR-LE vs. DP-HE; ¶DP-HE at 11.1 mmol/l glucose vs. DP-HE at 1.65 mmol/l glucose.

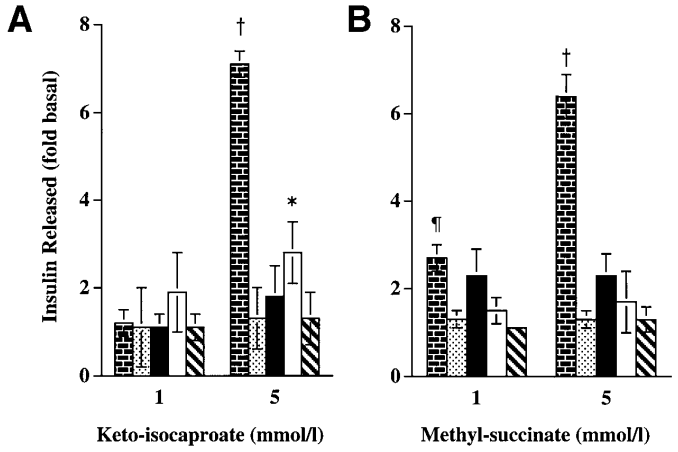

FIG. 2. Insulin release in response to mitochondrial substrates in *P. obesus* islets. Release rates are expressed as fold of basal insulin release (at 2.0 mmol/l glucose) during 1-h incubation. Glucose (2.0 mmol/l) was present during the incubation with KIC or monomethyl-succinate. The response of rat islets is given for comparison. Each point is the mean of 5–7 experiments ± SE. ▣, Rat; ▩, DR-LE; ■, DR-HE; □, DP-LE; ▨, DP-HE. *$P < 0.05$; ¶$P < 0.003$; †$P < 0.002$, all vs. basal insulin response.

DP-HE *P. obesus* islets, islets of the hyperglycemic animals were conditioned for 18 h in low (3.3 mmol/l) glucose supplemented with diazoxide (375 µmol/l) (see RESEARCH DESIGN AND METHODS). Previous studies have suggested that high ambient glucose levels lead to rapid depletion of islet insulin stores in *P. obesus* as a result of an increased secretory drive uncompensated for by the biosynthetic machinery of the islets (4,5,7,11). Given the shift to the left of the glucose-insulin dosage-response curve in *P. obesus* islets (7), we added diazoxide to the low-glucose–conditioned islets to further reduce the secretory drive, allowing prompt recovery of the islets from in vivo hyperglycemia. Islets of hyperglycemic DP-HE *P. obesus* maintained 18 h in low glucose and diazoxide exhibited improved insulin response to glucose (see below). Islets of DP-HE *P. obesus* maintained 18 h in low glucose without diazoxide showed a smaller insulin response to glucose, whereas conditioning in high (11.1 mmol/l) glucose had no effect (data not shown). Furthermore, an 18-h culture in low glucose and diazoxide led to no significant improvement in insulin response to KIC or to monomethyl succinate as well as in fractional glucose oxidation in islets of DP-HE *P. obesus* (data not shown). Hence 18 h of low ambient glucose failed to improve mitochondrial oxidative activity in islets of hyperglycemic *P. obesus*.

Insulin-potentiating pathways in *P. obesus* islets. The activity of insulin release–potentiating pathways in *P. obesus* β-cells was tested in static incubations. Glucagon-like peptide 1 (GLP-1; 50 nmol/l) and forskolin (5 µmol/l) were used to examine the effects of receptor- and receptor-independent activation of cyclic AMP–dependent protein kinase (PKA) on glucose-induced insulin release. Figure 3 shows that GLP-1 had no effect on basal insulin secretion in islets of DR-HE *P. obesus*, but nearly doubled glucose-stimulated (11.1 mmol/l) insulin release ($P < 0.04$). On the other hand, GLP-1 significantly potentiated both basal and glucose-stimulated insulin responses in islets from DP-LE or DP-HE *P. obesus*. Likewise, forskolin was ineffective in augmenting basal insulin release in islets of DR animals, but potentiated the glucose-stimulated response by more than threefold; in contrast, DP islets exhibited increased sensitivity to forskolin at basal glucose levels as well as in the glucose-stimulated state (Fig. 3). Thus it is clear that the PKA-dependent insulin release–amplifying pathway is functional in islets of *P. obesus*, with DP islets even exhibiting increased sensitivity compared to islets of DR *P. obesus*.

Activation of the lipid-dependent PKC is another potentiating pathway in pancreatic β-cells. Short-term exposure to low concentrations (0.1 µmol/l) of phorbol 12-myristate 13-acetate (PMA) results in the activation of most PKC isoenzymes. In islets obtained from DR *P. obesus* (Fig. 4*A*), the addition of PMA to the incubation media had minimal effect on nonstimulated (2.0 mmol/l glucose) insulin release and 1.2- and 3-fold potentiating effect on the glucose-stimulated (11.1 mmol/l) insulin response in islets from DR-LE and DR-HE animals, respectively ($P < 0.02$). In DP-LE *P. obesus* islets, PMA led to a 2.3- and 1.7- fold increased insulin release in basal

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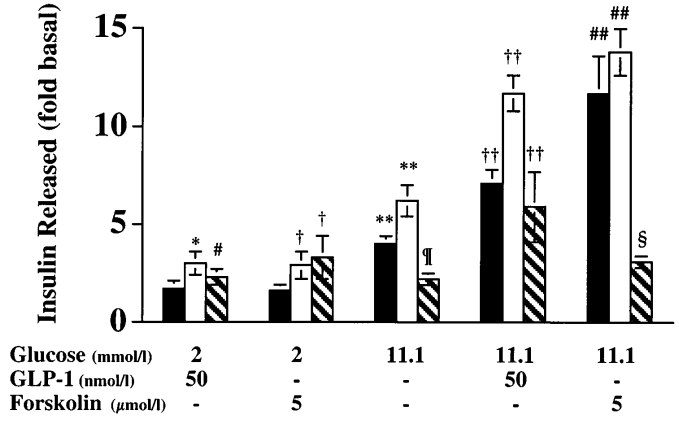

FIG. 3. Insulin release in response to GLP-1 and forskolin in *P. obesus* islets. Release rates are expressed as fold of basal insulin release (at 2.0 mmol/l glucose) during 1-h incubation. Each point is the mean of 5–6 experiments ± SE. ■, DR-HE; □, DP-LE; ◪, DP-HE. **P* < 0.02, #*P* < 0.01, †*P* < 0.05, ***P* < 0.002, ¶*P* < 0.005 vs. basal insulin response; ††*P* < 0.05, ##*P* < 0.003, §*P* < 0.02 vs. insulin response to 11.1 mmol/l glucose.

and glucose-stimulated conditions ($P < 0.02$ and $P < 0.005$), respectively. In contrast, islets of hyperglycemic DP-HE *P. obesus* showed no significant response to PMA at either basal or maximal stimulatory glucose concentration (Fig. 4*A*).

The possibility that hyperglycemia affects PKC-mediated potentiation of insulin response was next examined by maintaining islets of DP-HE *P. obesus* overnight at low glucose in the presence of diazoxide. Figure 4*B* shows that 18-h maintenance of islets from the hyperglycemic animals in low glucose dramatically improved the insulin response to both maximal stimulation with glucose and PMA: the response to 11.1 mmol/l glucose increased from 2.1 ± 0.3–fold in freshly isolated islets to 9.4 ± 1–fold in islets maintained in low glucose ($P < 0.005$) (compare Figs. 4*A* and *B*), and the response to PMA increased from 1.3 ± 0.2–fold to 4.4 ± 0.8–fold at basal glucose and from 2.3 ± 0.3–fold to 18.4 ± 1.7–fold at maximal glucose in fresh islets versus islets maintained 18 h in low glucose, respectively ($P < 0.005$ for both). These findings point toward a possible link between the deranged insulin response to glucose and PMA and a "toxic" effect of glucose on PKC-dependent signals.

Confocal imaging of β-cells in pancreatic sections from DR-HE, DP-LE, and DP-HE *P. obesus* enabled us to show a differential effect of hyperglycemia, diet, or line selection on the expression and localization of PKC isoenzymes. Representative images are shown in Fig. 5 and their relative intensities are quantified in Table 1. Levels of α-PKC were markedly diminished in islets of hyperglycemic (DP-HE) *P. obesus*, displaying a 50% reduction relative to islets of normoglycemic DP-LE *P. obesus* and 65% reduction relative to islets of DR-HE *P. obesus*. Small reduction in levels of λ-PKC were noted in islets of DP *P. obesus* relative to those of DR *P. obesus*. However, although islets of normoglycemic DP and DR *P. obesus* exhibited clear granular localization of the isoenzyme, indicative of receptor for activated C kinase (RACK)-bound–activated PKC (14), λ-PKC appeared mostly defuse in islets of DP-HE *P. obesus*, most likely in an inactive form, with minimal degree of granular localization. Reversed trends were observed in images of ζ-PKC; although ring-structured formations adjacent to the nuclear envelope were observed in islets from DR-HE *P. obesus* (Fig. 5), a five- to sixfold higher expression of ζ-PKC was observed in the DP animals (Table 1), the isoenzyme being concentrated in granulated structures throughout the β-cell. The expression of ε-PKC and θ-PKC appeared to be related to the animals' diet, as almost fourfold increased levels of both isoenzymes were observed in islets of DP-HE and DR-HE as compared with those of DP-LE *P. obesus* islets.

DISCUSSION

Human populations that have undergone rapid transition from a low caloric intake to richer diets display a high frequency of type 2 diabetes (15–17). One of the attractive aspects of the *P. obesus* as an animal model for type 2 diabetes is that its diabetic phenotype revealed itself during the process of adaptation from desert conditions of low caloric intake to the relative high-calorie diet of laboratory feed. As in human type 2 diabetes, two factors must be present in this model to express the diabetic phenotype: peripheral resistance to insulin, a characteristic found in all *P. obesus* regardless of their selection line (8), and inadequate β-cell capacity to release enough insulin to compensate for the increased caloric intake in the face of insulin resistance (4,5,7,11). A further advantage of this model is that two lines have been selected: the DP line, which develops hypergly-

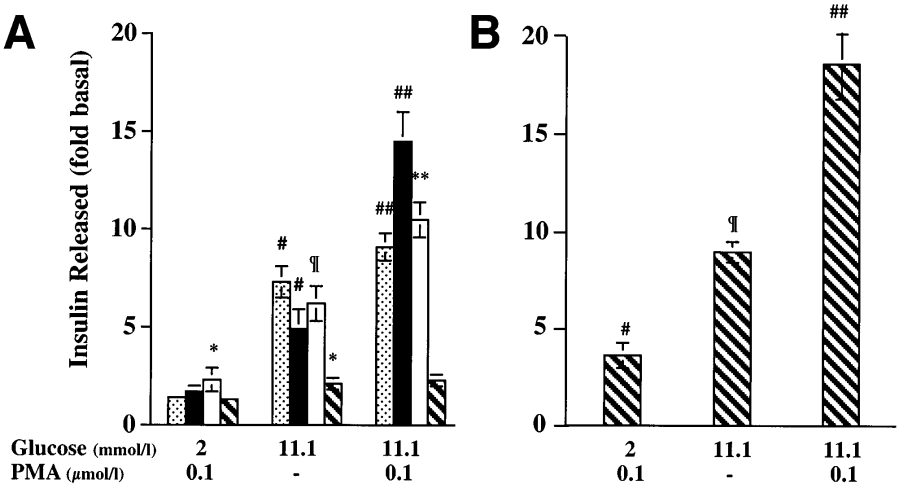

FIG. 4. Insulin release in response to glucose and PMA in freshly isolated *P. obesus* islets (*A*) and islets of hyperglycemic *P. obesus* cultured overnight in the presence of 3.3 mmol/l glucose and 375 μmol/l diazoxide (*B*). Release rates are expressed as fold of basal insulin release (at 2.0 mmol/l glucose) during 1-h incubation. Each point is the mean of 5–7 experiments ± SE. ▨, DR-LE; ■, DR-HE; □, DP-LE; ◪, DP-HE. **P* < 0.05, #*P* < 0.02, ¶*P* < 0.005 vs. basal insulin response; ##*P* < 0.02, ***P* < 0.05 vs. insulin response to 11.1 mmol/l glucose.

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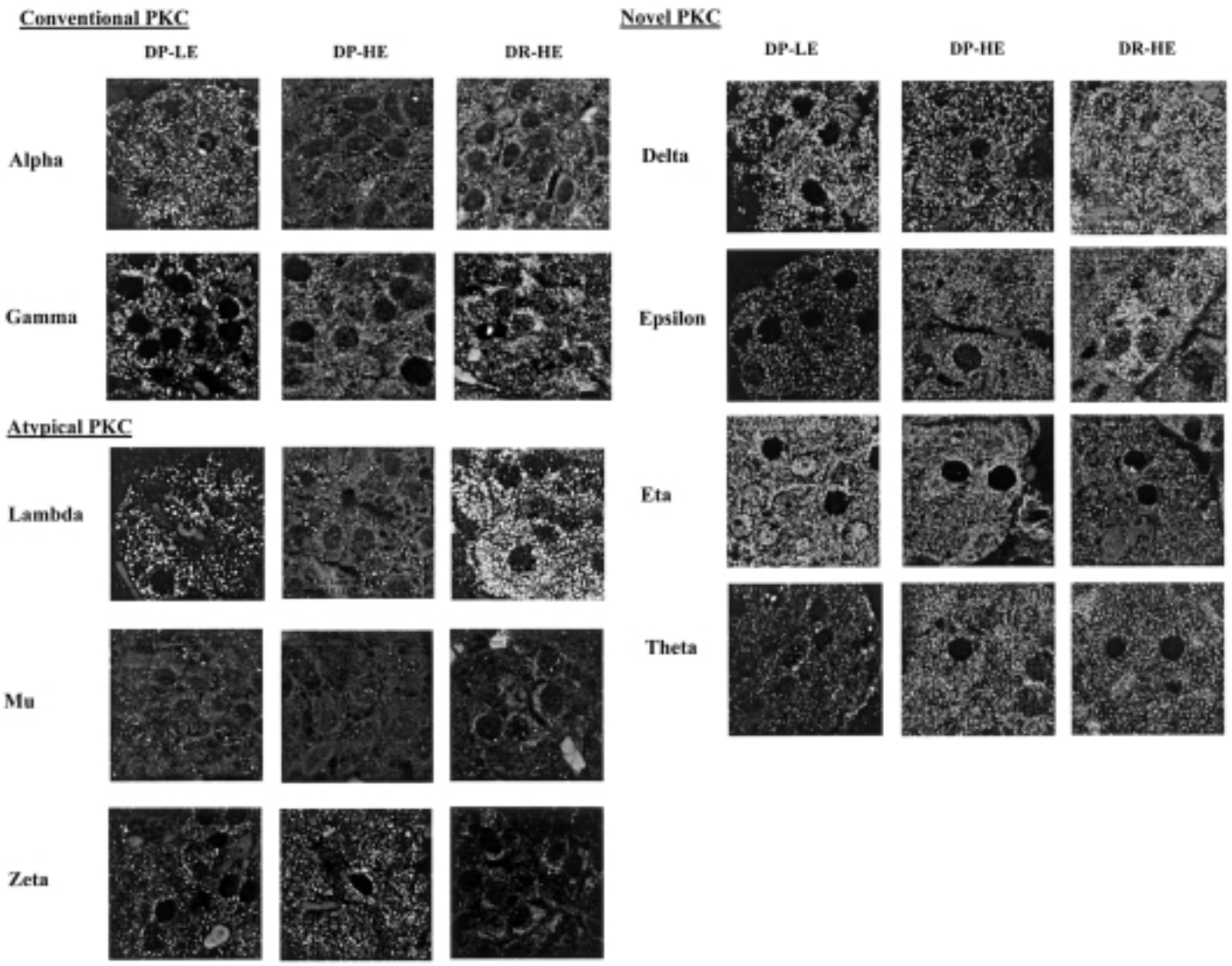

FIG. 5. In situ PKC isoenzyme expression in *P. obesus* β-cells. Pancreatic sections from hyperglycemic (DP-HE), prediabetic-normoglycemic (DP-LE), and diabetes resistant (DR-HE) *P. obesus* were immunostained with isoenzyme-specific antisera and image analyzed using laser-scanning confocal microscopy. Positive β-cell identification was achieved by counterstaining with insulin antiserum (not shown).

cemia within days of exposure to an HE diet, and the DR line, which is able to maintain normoglycemia despite sustained HE nutrition (3). Because a similar degree of resistance to insulin has been reported in both lines of *P. obesus* (8), differences in islet function were expected be the determining cause for diabetes evolution (18,19). Importantly, hyperglycemia and many of the defects associated with diminished β-cell function are readily reversible by removal of the dietary stress in *P. obesus* (4,7).

Information on β-cell processes related to the diminished capacity of *P. obesus* islets to adapt to food excess is limited. In previous studies, we have shown that under nutritional load, DP *P. obesus* islets released excessive amounts of proinsulin, concomitantly with drastic depletion of their insulin stores (4,5). Under these conditions, the islets exhibited a lower threshold for glucose, an occurrence that may partly be attributable to the increased hexokinase activity demonstrated in these islets (7). Furthermore, first-phase insulin release was deficient. In this study we extended these observations and examined the major pathways known to regulate insulin release in other species.

Glycolysis and mitochondrial oxidative activity are believed to be the primary pathways involved in initiating insulin release (20,21). Glycolytic rates in islets of normoglycemic (DR or prediabetic DP-LE) *P. obesus* at a maximal stimulatory concentration of glucose (11.1 mmol/l) were diminished, and corresponded to 30–40% of those reported in rat (22) or human islets (23). Islets of hyperglycemic (DP-HE) *P. obesus* exhibited rates twofold higher than those seen in normoglycemic controls (Fig. 1), a finding compatible with the demonstration of increased capacity to phosphorylate glucose under these conditions (7). Although rat islets were reported to oxidize 30–43.7% of glucose utilized at the maximally stimulatory concentration of hexose (9,22), *P. obesus* islets oxidized only 14–22% of glucose entering that pathway. Taken together with the lower rates of glucose utilization, these findings indicate very low absolute glucose oxidation rates in *P. obesus*. Furthermore, in islets from hyperglycemic *P. obesus*,

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TABLE 1
Relative expression of PKC isoenzymes in pancreatic β-cells of *P. obesus*

Isoenzyme	DP-LE	DP-HE	DR-HE
Conventional PKC			
α	65	33*	100
γ	82	80	100
Atypical PKC			
λ	57	70	100
μ	128	83	100
ζ	564*	483*	100
Novel PKC			
δ	88	94	100
ϵ	25*	103	100
η	139	128	100
θ	13*	74	100

Localization and levels of PKC isoenzymes were determined on immunohistochemical images of pancreatic sections obtained from DP-LE, DP-HE, and DR-HE, as depicted in Fig. 5, and expressed as percent values obtained in DR-HE islets. β-cells were identified by counterstaining with anti-insulin antibody. Preset scanning conditions were maintained for each isoenzyme. *Twofold differences in image density were considered significant.

fractional glucose oxidation declined from 22.6 ± 1.5 to 13.0 ± 0.9% when the concentration of glucose was increased from 1.7 to 11.1 mmol/l (Fig. 1*C*). Glucose oxidation in islets of other models of type 2 diabetes is mostly lower than their corresponding controls, but exceptions have been reported. Although the glucose oxidation rates of neonatal streptozotocin-induced diabetic rats have been reported to be lower than those of control rat islets (24,25), age-dependent variations have been reported in colonies of the GK rat. In the St. Mary Hospital colony in London, glucose oxidation was similar in islets of GK and control Wistar rats at age 8 weeks, but GK rats' glucose utilization rates were higher (26); at age 14 weeks, the rates of glucose utilization and oxidation were similar between the groups (27). At the Karolinska Institute in Stockholm, islets of the GK colony (8–12 weeks old) demonstrated an increase in rates of glucose utilization and oxidation as compared with islets of Wistar rats, resulting in similar fractional glucose oxidation (28). Finally, islets obtained from 60% depancreatized rats revealed increased rates of glucose oxidation 2 weeks after operation relative to islets of sham-operated controls (29). Thus *P. obesus* islets appear to have a low intrinsic oxidative capacity relative to other species, which may be appropriate for the animal's low-energy nutrition in the wild.

The inability of DP-HE islets to increase glucose oxidation and the overall low fraction of glucose oxidized are indicative of inadequate mitochondrial function. This was further corroborated by the demonstrated lack of insulin response to KIC or monomethyl succinate, both exclusive mitochondrial substrates in pancreatic β-cells, in contrast to the six- to sevenfold increase in insulin response to either secretagogue in rat islets. These findings warrant further careful investigation. One possibility is that *P. obesus* islets contain a low number of mitochondria or have mitochondria with a species-related defect. Using a highly effective inhibitor of fatty acid oxidation, we ruled out preferential oxidation of fat as a reason for diminished glucose oxidation in hyperglycemic *P. obesus* islets. Whatever the cause, low mitochondrial capacity to oxidize nutrients would lead to insufficient ATP production. ATP is needed for multiple steps involved in insulin production, storage, and release, especially in the presence of caloric load; ATP deficiency may explain both depletion of insulin content and deranged dynamics of insulin release in DP *P. obesus* islets (7). The twofold higher amount of glucose utilized in islets of DP-HE *P. obesus* as compared with islets from normoglycemic animals was most likely shuttled to lactate.

Normal kinetics and the magnitude of the insulin response to glucose necessitate that both the initial signal for stimulus-secretion coupling and the potentiating signals are intact in the β-cell (21). The adenylyl cyclase–cyclic AMP-PKA coupling system and the lipid-dependent PKC coupling system represent two major gain amplifiers that ascribe glucose competence to the β-cell (21,30); their functions were therefore tested in *P. obesus* islets. Activation of PKA by GLP-1, a receptor ligand, or by forskolin, which maximally activates adenylyl cyclase, bypassing the receptor and the coupling G proteins, resulted in significant potentiation of the insulin response to glucose in *P. obesus* islets regardless of line or diet (Fig. 3). In the presence of low (2.0 mmol/l) glucose, islets of DP *P. obesus* responded to stimulation by both GLP-1 and forskolin, whereas islets of DR *P. obesus* did not. This increased sensitivity to PKA activation in the DP line at low glucose may be related to the increased phosphorylation capacity previously observed in DP *P. obesus* (7), as well as to increased β-cell glycogen stores (31), which may be mobilized for fuel by the activated PKA. Thus, despite the marked reduction in insulin content (>90%) (7), islets of hyperglycemic *P. obesus* responded to PKA stimulation of secretion like normal rat islets. On the other hand, the PKC-dependent stimulus-amplifying branch seemed to be diminished in *P. obesus* in a glucose-dependent fashion. When tested using PMA, a nonselective irreversible activator of most isoforms of PKC, islets of hyperglycemic *P. obesus* exhibited no response, in contrast to the adequate response observed in islets of normoglycemic prediabetic DP or DR animals (Fig. 4). Subjecting islets of hyperglycemic *P. obesus* to low ambient glucose in vitro fully restored the response to PKC activation and dramatically improved the insulin response to glucose. These observations suggest that the activity of one or more PKC isoforms is diminished during induction of hyperglycemia in the DP line, with an 18-h culture in low glucose being sufficient to restore that activity, and that the diminished PKC activity may be partly responsible for the poor insulin response to glucose in this line. Digitized immunohistochemical imaging provided additional evidence for hyperglycemia-related diminished expression of selected PKC isoenzymes (Fig. 5), revealing reduced expression of α-PKC and λ-PKC in β-cells of DP-HE *P. obesus* as compared with β-cells of normoglycemic DR-HE or DP-LE animals. The role of individual PKC isoenzymes in β-cell signal transduction is poorly defined. A role for α-PKC in glucose-induced insulin response in rat islets was first suggested by Rasmussen and coworkers (32–34). Using selective RACK-binding inhibitors, we extended these findings and showed that α-PKC and ε-PKC are involved in glucose-induced insulin response in rat islets (10); however, their target substrates and precise roles are still unclear. Because no significant changes were observed in levels or localization of ε-PKC

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in hyperglycemic *P. obesus*, it stands to reason that the reduced levels of α-PKC are an important factor responsible for the diminished insulin response to PMA and glucose in this animal. No information is available on the function of λ-PKC in pancreatic β-cells. Therefore, whether the observed diminished translocation/activation of the atypical λ-PKC is also related to loss of glucose responsiveness in hyperglycemic *P. obesus* awaits further clarification.

Imaging the novel ε-PKC and θ-PKC in β-cells of *P. obesus* revealed dramatic diet-dependent increased expression, independent of the animal's tendency to develop diabetes. When fed the HE diet, animals of either line displayed five- to sevenfold higher levels of the two isoenzymes. Although ε-PKC has been shown to play a role in glucose-induced calcium-independent insulin response (10), the significance of an HE diet–induced increased expression of both ε-PKC and θ-PKC awaits further studies. In contrast, the DP *P. obesus* on either diet exhibited significant increase and granule-like aggregation of ζ-PKC relative to β-cells of DR *P. obesus*. Thus it remains to be seen whether altered levels and/or localization of ζ-PKC contributes to the animal's tendency to develop diabetes.

In summary, our data suggest that deficient mitochondrial oxidation of glycolytic products may be the biochemical basis for the β-cell defects in *P. obesus* that eventually lead to nutrition-induced diabetes in this species. The concomitant hyperglycemia-induced reduction of α-PKC (and possibly of λ-PKC) activities may contribute to the deficient insulin responsiveness to glucose.

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