Increasing Triglyceride Synthesis Inhibits Glucose-Induced Insulin Secretion in Isolated Rat Islets of Langerhans: A Study Using Adenoviral Expression of Diacylglycerol Acyltransferase

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The mechanisms by which prolonged exposure to elevated levels of fatty acids (FA) adversely affects pancreatic β-cell function remain unclear. Studies in the Zucker diabetic fatty rat have suggested that excessive accumulation of triglycerides (TG) in islets plays a key role in the deleterious effects of FA. However, a direct relationship between TG accumulation and defective β-cell function has not been established. The aim of the present study was therefore to determine whether increasing TG synthesis in isolated rat islets of Langerhans impairs insulin secretion. To this end, we infected isolated rat islets with an adenovirus encoding for the enzyme catalyzing the last step of triglyceride synthesis, acyl-coenzyme Adiacylglycerol acyltransferase 1 (DGAT). DGAT overexpression did not modify glucose oxidation nor palmitate oxidation, but increased palmitate incorporation into triglycerides by approximately 2-fold. Islets overexpressing DGAT and cultured in elevated glucose levels for 72 h had markedly impaired insulin secretion in response to glucose, but responded normally to the nonglucose secretagogues glyburide and potassium chloride. The deleterious effects of DGAT overexpression were not additive to those of prolonged exposure to palmitate. We conclude that a selective increase in TG content impairs glucose-induced insulin secretion, a mechanism likely to mediate, at least in part, the deleterious effects of FA on pancreatic β-cell function. (Endocrinology 143: 3326–3332, 2002)

PROLONGED exposure to elevated levels of fatty acids (FA) adversely affects pancreatic β-cell function, a phenomenon referred to as lipotoxicity (1, 2). Most of the studies of lipotoxicity have been carried out in a massively obese rodent model of diabetes, the Zucker diabetic fatty (ZDF) rat. Male ZDF rats develop overt diabetes between 7 and 10 wk of age. The occurrence of diabetes is associated with a dramatic increase in islet triglyceride (TG) content (3, 4). Increased intracellular TG content has initially been hypothesized to be the cause of β-cell dysfunction in these animals, because TG depletion through caloric restriction, troglitazone, or leptin administration is associated with reversal of the diabetic phenotype (5–7). Further studies by the same group have implicated intracellular ceramide synthesis in the diabetic phenotype (5–7). However, the relevance of observations made in ZDF rat islets has not been demonstrated. The aim of the present study was to directly ascertain whether increasing TG synthesis impairs glucose-induced insulin secretion and gene expression. To this end, we used an adenovirus to overexpress the only dedicated enzyme in TG synthesis, acyl-coenzyme A (acyl-CoA)/diacylglycerol acyltransferase 1 (DGAT; EC 2.3.1.20), in isolated rat islets.

Materials and Methods

Animals

Six-week-old male Wistar rats were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Animals were housed on a 12-h light, 12-h dark cycle with free access to water and standard laboratory chow. All procedures using animals were approved by the Pacific Northwest Research Institute institutional animal care and use committee.

Generation of adenoviruses

A 1.6-kb XhoI/XbaI fragment of the mouse DGAT cDNA with an N terminal FLAG epitope was excised from plasmid pBSSK (gift from Dr. Robert V. Farese, Jr., Gladstone Institute of Cardiovascular Disease) and subcloned into pAdTrack-CMV (14). Pmel-linearized pAdTrack-CMV-DGAT was then transformed into Escherichia coli BJ5183 cells along with the adenoviral vector pAdEasy for bacterial recombination. Recombinant adenoviruses (Ad-DGAT) were produced in 293 cells and purified. Generation of adenoviral vectors was described previously (14). The deleterious effects of DGAT overexpression were not additive to those of prolonged exposure to palmitate. We conclude that a selective increase in TG content impairs glucose-induced insulin secretion, a mechanism likely to mediate, at least in part, the deleterious effects of FA on pancreatic β-cell function. (Endocrinology 143: 3326–3332, 2002)

Abbreviations: Ad-Luc, Firefly luciferase-expressing adenovirus; CoA, coenzyme A; DG, diglycerides; DGAT, diacylglycerol acyltransferase 1; FA, fatty acids; FBS, fetal bovine serum; KRB, Krebs-Ringer buffer; LC-CoA, long-chain fatty acyl-coenzyme A; pfu, plaque-forming units; TG, triglyceride; ZDF, Zucker diabetic fatty.
Rat islet isolation and culture

Rat islets were isolated by collagenase digestion as previously described (12). After an overnight culture in RPMI 1640 containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 11.1 mM glucose to ensure optimal recovery (16), batches of 100–200 islets were infected with 104 plaque-forming units (pfu)/islet of Ad-Luc or Ad-DGAT for 1 h unless otherwise indicated. After centrifugation for 10 min at 1200 rpm, islets were resuspended in fresh medium and incubated in various experimental conditions as described in Results. Preparation of culture medium containing palmitate was previously described (12). The final molar ratio of palmitate/BSA was 5:1. All control conditions contained the same amount of BSA and vehicle (ethanol/H2O, 1:1) as those with palmitate.

Immunoblotting

Islets were harvested in 75 μl lysis buffer [140 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM CaCl2, 1 mM MgCl2, 10% glycerol, 1% Nonidet P-40, 1 mM NaN3, 0.5 mM phenylmethylsulfonylfluoride, 0.5 mM dithiothreitol, and 1 μg/ml each of leupeptin, aprotinin, antipain, and pepstatin A], and frozen and thawed three times. Cell debris was removed by centrifugation. Twenty micrograms of protein were separated by SDS-PAGE with 8% acrylamide gels. Resolved proteins were transferred to nitrocellulose membranes (Bio-Rad Laboratories, Inc., Hercules, CA) by electroblotting. The membranes were blocked with 1% nonfat dry milk in PBS with 0.05% Tween for 1 h and then incubated for 1 h at room temperature with a mouse monoclonal anti-FLAG antibody incubated in various experimental conditions as described in Results. Detection of the appropriate apparent molecular weight was detected by immunoblotting with an anti-FLAG antibody. A representative blot is shown in Fig. 1B, lanes 1–4.

Expression of data and statistics

Data are expressed as the mean ± SE. Intergroup comparisons were performed by paired t test or ANOVA with post hoc Bonferroni adjustment where appropriate. P < 0.05 was considered significant.

Results

Overexpression of DGAT in islets

Isolated islets were infected for 1 h in the presence of 0, 106, or 108 pfu/islet of Ad-DGAT, and then cultured for 24 h in RPMI 1640 containing 10% FBS and 11.1 mM glucose. The expression of DGAT mRNA was examined by fluorescence-based RT-PCR. As shown in Fig. 1A, the amount of DGAT mRNA increased with the virus titer and was readily detectable at concentrations of 106 and 108 pfu/islet. Next, isolated islets were infected under the same conditions at concentrations of 105, 106, and 107 pfu/islet, and the expression of DGAT protein was detected by immunoblotting using the anti-FLAG antibody (Fig. 1B, lanes 1–4). A band of the appropriate apparent molecular weight was detected at 37 C. Islets were then washed and incubated in KRB containing 0.1% BSA, 2.8 or 16.7 mM glucose, 0.5 μCi/μmol [1-14C]palmitate, 0.1 mM unlabeled palmitate, and 1 mM carnitine for 1 h at 37 C. The production of 14CO2 in the buffer was determined as described above. Islets were harvested at the end of the final incubation for lipid extraction and thin layer chromatographic analysis as previously described (12).

Glucose oxidation

Batches of 100 isolated islets each were preincubated twice for 30 min at 37 C in KRB containing 0.1% BSA and then incubated in KRB containing 0.1% BSA, 0.1 μCi/μmol [U-14C]glucose (NEN Life Science Products, Boston, MA), and either 2.8 or 16.7 mM unlabeled glucose for 1 h at 37 C. One milliliter of incubation buffer was then transferred to an Erlenmeyer with a rubber cap and acidified with 100 μl 7% perchloric acid. Benzethonium hydroxide (400 μl) was then injected into small wells suspended to the rubber caps, and after 16 h at room temperature, trapped 14CO2 was measured by liquid scintillation counting. Background counts from a control condition treated side by side with the samples but without islets was subtracted from the counts.

Palmitate oxidation and esterification

Batches of 100 isolated islets each were prelabelled overnight in RPMI 1640 containing 11.1 mM glucose, 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.1% BSA, 0.5 μCi/μmol [1-14C]palmitate (NEN Life Science Products), 0.1 mM unlabeled palmitate, and 1 mM carnitine.
Effects of DGAT overexpression on glucose and palmitate metabolism

The effects of DGAT overexpression on FA esterification were assessed by measuring incorporation of $^{14}$C-labeled palmitate into TG, diglycerides (DG), and phospholipids (Fig. 2). Islets overexpressing DGAT incorporated less palmitate into phospholipids (Fig. 2A; $P < 0.01$; $n = 5$) and twice as much palmitate into TG (Fig. 2B; $P < 0.01$; $n = 5$) than islets infected with Ad-Luc. Incorporation of palmitate into DG was unchanged (Fig. 2B; $P = NS$; $n = 5$). The rates of glucose and palmitate oxidation in islets infected with Ad-Luc or Ad-DGAT are shown in Table 1. As expected, the rate of glucose oxidation was higher at 16.7 mM glucose than at 2.8 mM glucose in both Ad-DGAT-infected ($P < 0.05$; $n = 5$) and Ad-Luc-infected ($P < 0.05$; $n = 5$) islets. However, DGAT overexpression had no effect on glucose oxidation at either 2.8 mM ($P = NS$; $n = 5$) or 16.7 mM ($P = NS$; $n = 5$) glucose. Also as expected, glucose inhibited palmitate oxidation in both Ad-DGAT-infected ($P < 0.05$; $n = 4$) and Ad-Luc-infected ($P < 0.05$; $n = 4$) islets. However, the rate of palmitate oxidation was similar in Ad-DGAT- and Ad-Luc-infected islets at both 2.8 mM ($P = NS$; $n = 4$) and 16.7 mM ($P = NS$; $n = 4$) glucose. Overall, these results indicate that DGAT overexpression selectively increased the flux through the FA esterification pathway and TG synthesis without affecting the mitochondrial oxidation of either glucose or palmitate.

**TABLE 1. Effects of DGAT overexpression on glucose and palmitate oxidation in islets**

<table>
<thead>
<tr>
<th>Glucose (mM)</th>
<th>2.8</th>
<th>16.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose oxidation (pmol CO$_2$/islet/h)</td>
<td>13.0 ± 2.5</td>
<td>40.8 ± 8.1*</td>
</tr>
<tr>
<td>Ad-Luc (n = 5)</td>
<td>15.0 ± 2.9</td>
<td>34.1 ± 8.8*</td>
</tr>
<tr>
<td>Ad-DGAT (n = 5)</td>
<td>9.7 ± 1.4</td>
<td>5.3 ± 1.2*</td>
</tr>
<tr>
<td>Palmitate oxidation (pmol CO$_2$/islet/h)</td>
<td>8.7 ± 1.4</td>
<td>4.6 ± 1.2*</td>
</tr>
<tr>
<td>Ad-Luc (n = 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad-DGAT (n = 4)</td>
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Glucose and palmitate oxidation in islets were measured as the production of $^{14}$CO$_2$ from labeled glucose or palmitate, respectively, after 1 h of incubation (preceded by 24 h of prelabeling in the case of palmitate), as described in Materials and Methods. Glucose oxidation was greater at 16.7 than at 2.8 mM glucose, as expected. Palmitate oxidation was decreased approximately 50% in the presence of 16.7 mM glucose compared with 2.8 mM glucose. DGAT overexpression had no effect on the rate of glucose or palmitate oxidation.

* $P < 0.05$ vs. 2.8 mM glucose.

Fig. 2. Isolated rat islets were infected for 1 h with 10$^9$ pfu/islet of Ad-DGAT or Ad-Luc, then cultured overnight in the presence of [1-$^{14}$C]palmitate, 0.1 mM unlabeled palmitate, and 11.1 mM glucose. Incorporation of labeled palmitate into cellular lipids was assessed by thin layer chromatography as described in Materials and Methods. A. Incorporation into phospholipids. B. Incorporation into triglycerides and diglycerides. Results are expressed as picomoles of palmitate incorporated per islet and are the mean ± SE of five replicate experiments. * $P < 0.05$. 

**Effects of DGAT on insulin secretion**

Ad-DGAT- or Ad-Luc-infected islets were cultured for 72 h in either 2.8 or 16.7 mM glucose, and insulin secretion was assessed in static incubation (Fig. 3). Insulin secretion after culture in 2.8 mM glucose was similar between Ad-DGAT-infected and Ad-Luc-infected islets (Fig. 3A). In contrast, insulin secretion in response to 8.3 mM ($P < 0.05$; $n = 5$) and 16.7 mM ($P < 0.001$; $n = 8$) glucose was significantly decreased in islets overexpressing DGAT after culture in 16.7 mM glucose (Fig. 3B). Insulin secretion in response to glyburide or potassium chloride, however, was not affected by DGAT overexpression (both $P = NS$; $n = 5$). DGAT overexpression did not affect insulin secretion after 24 h of expression of the enzyme (data not shown).
palmitate in the culture medium led to a dose-dependent decrease in the subsequent response to 16.7 mM glucose. In contrast, islets infected with Ad-DGAT showed a reduced response to 16.7 mM glucose even in the absence of palmitate (*, *P < 0.05; **, *P < 0.001), confirming the results shown in Figs. 3 and 4, and the response was not further impaired with increasing amounts of palmitate. These results suggest a common mechanism of action between palmitate exposure and DGAT overexpression, which might lie at the level of TG accumulation.

Effects of DGAT on insulin mRNA levels

To determine the effects of DGAT overexpression on insulin mRNA levels, islets infected with Ad-DGAT or Ad-Luc were cultured for 72 h in the presence of 2.8 mM glucose, 16.7 mM glucose, or 16.7 mM glucose and 0.5 mM palmitate. Insulin and β-actin mRNA levels were assessed by fluorescence-based RT-PCR (Fig. 6). As expected, insulin mRNA levels were higher in islets cultured in high glucose than in islets cultured in low glucose (*, *P < 0.01; **, *P < 0.001). However, DGAT overexpression did not affect insulin mRNA levels in any of the culture conditions (*P = NS; n = 3). Thus, this study was designed to assess whether selectively increasing FA esterification by overexpressing the TG-synthesizing enzyme DGAT impairs glucose-induced insulin secretion in isolated rat islets. Our results show that DGAT overexpression reduces the secretion of insulin in response to glucose after prolonged culture in elevated glucose levels.

Discussion

This study was designed to assess whether selectively increasing FA esterification by overexpressing the TG-synthesizing enzyme DGAT impairs glucose-induced insulin secretion in isolated rat islets. Our results show that DGAT overexpression reduces the secretion of insulin in response to glucose after prolonged culture in elevated glucose levels.

Fig. 3. Isolated rat islets were infected with 10⁸ pfu/islet of Ad-DGAT or Ad-Luc for 1 h and cultured for 72 h in the presence of 2.8 mM (A) or 16.7 mM (B) glucose. Insulin secretion was assessed in 1-h static incubations in response to 2.8 mM (n = 8), 8.3 mM (n = 5), or 16.7 mM (n = 8) glucose; 2.8 mM glucose plus 10 mM glyburide (n = 5); or 2.8 mM glucose plus 40 mM potassium chloride (n = 5). Results are the mean ± SE of six replicate experiments. *, *P < 0.05; **, *P < 0.001.

Fig. 4. Isolated rat islets were infected with 10⁸ pfu/islet of Ad-DGAT or Ad-Luc for 1 h and cultured for 72 h in the presence of 16.7 mM glucose with or without 0.5 mM palmitate. Control conditions contained the same amount of BSA and vehicle (ethanol/H₂O, 1:1) as those with palmitate. Insulin secretion was assessed in 1-h static incubations in response to 2.8 or 16.7 mM glucose. Results are mean ± SE of six replicate experiments. *, *P < 0.01; **, *P < 0.001.

Fig. 5. Isolated rat islets were infected with 10⁸ pfu/islet of Ad-DGAT or Ad-Luc for 1 h and cultured for 72 h in the presence of 16.7 mM glucose and 0, 0.1, 0.25, or 0.5 mM palmitate. Control conditions contained the same amount of BSA and vehicle (ethanol/H₂O, 1:1) as those with palmitate. Insulin secretion was assessed in 1-h static incubations in response to 2.8 or 16.7 mM glucose. Results are mean ± SE of six replicate experiments. *, *P < 0.01; **, *P < 0.001.
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FIG. 6. Isolated rat islets were infected with 10⁶ pfu/islet of Ad-DGAT or Ad-Luc for 1 h and cultured for 72 h in the presence of 2.8 mM glucose, 16.7 mM glucose, or 16.7 mM glucose and 0.5 mM palmitate. Control conditions contained the same amount of BSA and vehicle (ethanol/H₂O, 1:1) as that with palmitate. Insulin and β-actin mRNA levels were compared by fluorescence-based RT-PCR as previously described (12). Results are normalized to insulin mRNA levels in Ad-Luc-infected islets cultured in 2.8 mM glucose and are mean ± SE of three replicate experiments.

Glucose (mM)

- 2.8
- 16.7
- 16.7 + Palmitate

Insulin/β-actin mRNA (% of 2.8 G)

Ad-Luc Ad-DGAT

FA; when both glucose and FA are chronically elevated, sustained inhibition of carnitine-palmitoyl transferase-1 by malonyl-CoA induces a switch in the partitioning of LC-CoA toward complex lipid synthesis. Consequently, deleterious effects of prolonged exposure to FA are only observed in the presence of elevated glucose levels, a hypothesis supported by several lines of experimental evidence (reviewed in Ref. 33). In a previous study we have demonstrated that accumulation of TG in isolated rat islets upon prolonged exposure to elevated FA occurs only in the presence of high glucose (12). We observed an inverse correlation between TG accumulation and insulin mRNA levels, suggesting that the functional effects of prolonged FA might be due to TG accumulation, as proposed in the ZDF rat (3, 4). However, to our knowledge a direct cause and effect relationship between TG accumulation and β-cell dysfunction has never been established, which was the objective of the present study.

DGAT catalyzes the last and only committed step in TG synthesis. It is a microsomal enzyme whose acyltransferase activity is highly specific for diacylglycerol (34) and which we have shown to be expressed in isolated islets and insulin-secreting cells (12). Validation of the DGAT gene in mice dramatically reduces diacylglycerol acyltransferase activity in several tissues, although the animals are still capable of some degree of TG synthesis. Indeed, an additional diacylglycerol acyltransferase gene family, DGAT2, has recently been identified (35, 36). DGAT overexpression in islets resulted in a 2-fold increase in the incorporation of palmitate into TG, without any detectable change in the rate of oxidation of either palmitate or glucose. Glucose-induced insulin secretion was unchanged after 24 h of expression of DGAT; however, it was significantly decreased after 72 h of expression in the presence of elevated glucose levels. The requirement for long-term culture for DGAT overexpression to affect insulin secretion is not due to delayed expression of the protein, which was already maximal after 24 h. It suggests that either several days in culture are necessary to accumulate enough TG to affect insulin secretion or that the effects of TG accumulation are only observed after 72 h in culture. It is interesting to note that exposure of islets to palmitate also requires 72 h to affect β-cell function (17). The glucose-dependent, long-term effects of DGAT thus have characteristics similar to the effects of palmitate on β-cell function (17). The glucose dependency of the DGAT effects are also consistent with the concept that FA-induced β-cell dysfunction is only observed in the presence of high glucose. Interestingly, insulin secretion in response to the nonnutrient secretagogues glyburide and potassium chloride were not perturbed by DGAT expression.

Thus, the effects of DGAT overexpression on glucose-induced insulin secretion share key characteristics with those of chronic FA: they only occur after prolonged culture (72 h), and they require the presence of elevated glucose levels during the culture. Most importantly, the effects of DGAT are not additive to those of palmitate. These findings therefore suggest that one mechanism by which prolonged exposure to FA diminishes insulin secretion is an increase in the esterification pathway and intracellular accumulation of TG. This does not prove, however, that accumulation of TG by itself is the mechanism underlying the effects of FA. In fact,
the concept that TG can cause β-cell dysfunction is somewhat counterintuitive, because storage of fat in the form of inert neutral lipids is usually seen as a defense mechanism by which the cell protects itself from the deleterious effects of FA (37). An alternative possibility is that the effects of FA are mediated by generation of an intermediate signal arising from the esterification pathway, such as DG.

Interestingly, we did not observe any effect of DGAT overexpression on insulin mRNA levels. This is in contrast to our previous observations that prolonged exposure to palmitate decreases insulin mRNA levels in islets in the presence of high glucose (12, 17). Although the reasons for the discrepancy between the effects of palmitate and those of DGAT expression on insulin gene expression are unknown, we speculate that exposure to palmitate is associated with multiple abnormalities in intracellular metabolism, none of which is mimicked by DGAT overexpression. Indeed, our results suggest that TG accumulation is a mechanism for FA-induced impairment of insulin secretion, but might not play a direct causal role in the FA-induced decrease in insulin gene expression. It is conceivable, for instance, that ceramide formation, which occurs upon prolonged exposure to FA (8) impairs insulin gene expression without affecting insulin secretion. Indeed, culture of insulin-secreting cells in the presence of cell-permeable ceramide analogs for 72 h does not impair insulin release (38). In addition, it is important to note that the present study examined the involvement of TG synthesis in defective insulin secretion, which might be one of the early signs of lipotoxicity, but not its potential role in more profound FA-induced abnormalities, such as β-cell death.

In conclusion, our results uniquely demonstrate that selectively increasing TG synthesis in the presence of elevated glucose levels impairs glucose-induced insulin secretion, supporting the concept that an increase in FA esterification in the presence of high glucose is a key mechanism of lipotoxicity. Whether these in vitro observations over relatively short periods of culture reflect the mechanisms of FA-induced β-cell dysfunction over many years of exposure of islets to elevated lipid levels in type 2 diabetic patients remains to be determined.

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